CHAPTER 11

Biotechnology : Principles and Processes

11.1 Principles of Biotechnology

1. The DNA molecule to which the gene of interest is integrated for cloning is called
(a) template (b) carrier
(c) transformer (d) vector. *(2015)*

2. The cutting of DNA at specific locations became possible with the discovery of
(a) selectable markers (b) ligases
(c) restriction enzymes (d) probes. *(2015)*

3. Which one of the following is a case of wrong matching?
(a) Somatic hybridization – Fusion of two diverse cells
(b) Vector DNA – Site for *t*RNA synthesis
(c) Micropropagation – *In vitro* production of plants in large numbers
(d) Callus – Unorganised mass of cells produced in tissue culture *(2012)*

4. Which one of the following techniques made it possible to genetically engineer living organisms?
(a) Recombinant DNA techniques
(b) X-ray diffraction
(c) Heavier isotope labelling
(d) Hybridization *(Mains 2011)*

5. Which of the following are used in gene cloning?
(a) Nucleoids (b) Lomasomes
(c) Mesosomes (d) Plasmids *(2010)*

6. Manipulation of DNA in genetic engineering became possible due to the discovery of
(a) restriction endonuclease
(b) DNA ligase
(c) transcriptase
(d) primase. *(2002)*

7. The bacteria generally used for genetic engineering is
(a) *Agrobacterium* (b) *Bacillus*
(c) *Pseudomonas* (d) *Clostridium*. *(2000)*

8. Which of the following is related to genetic engineering?
(a) Heterosis (b) Mutation
(c) Plastid (d) Plasmid *(1999)*

9. Genetic engineering is possible, because
(a) we can cut DNA at specific sites by endonucleases like DNase I
(b) restriction endonucleases purified from bacteria can be used *in vitro*
(c) the phenomenon of transduction in bacteria is well understood
(d) we can see DNA by electron microscope. *(1998)*

10. When scientists make an animal superior by view of genotype, introducing some foreign genes in it, is called
(a) immunization (b) genetic engineering
(c) tissue culture (d) biotechnology. *(1996)*

11. Which of the following organelles is related with genetic engineering?
(a) Mitochondria (b) Plasmids
(c) Golgi bodies (d) Lysosomes *(1994)*

11.2 Tools of Recombinant DNA Technology

12. Identify the wrong statement with regard to restriction enzymes.
(a) Each restriction enzyme functions by inspecting the length of a DNA sequence.
(b) They cut the strand of DNA at palindromic sites.
(c) They are useful in genetic engineering.
(d) Sticky ends can be joined by using DNA ligases. *(NEET 2020)*

13. Choose the correct pair from the following.
(a) Ligases - Join the two DNA molecules
(b) Polymerases - Break the DNA into fragments
(c) Nucleases - Separate the two strands of DNA
(d) Exonucleases - Make cuts at specific positions within DNA *(NEET 2020)*

14. The specific palindromic sequence which is recognised by *Eco*RI is
(a) 5′ - GAATTC - 3′ (b) 5′ - GGAACC - 3′
3′ - CTTAAG - 5′ 3′ - CCTTGG - 5′
(c) 5′ - CTTAAG - 3′ (d) 5′ - GGATCC - 3′
3′ - GAATTC - 5′ 3′ - CCTAGG - 5′.
(NEET 2020)

15. The sequence that controls the copy number of the linked DNA in the vector, is termed
(a) selectable marker (b) Ori site
(c) palindromic sequence (d) recognition site.
(NEET 2020)

16. In gel electrophoresis, separated DNA fragments can be visualized with the help of
(a) acetocarmine in bright blue light
(b) ethidium bromide in UV radiation
(c) acetocarmine in UV radiation
(d) ethidium bromide in infrared radiation.
(NEET 2020)

17. Following statements describe the characteristics of the enzyme restriction endonuclease. Identify the incorrect statement.
(a) The enzyme recognises a specific palindromic nucleotide sequence in the DNA.
(b) The enzyme cuts DNA molecule at identified position within the DNA.
(c) The enzyme binds DNA at specific sites and cuts only one of the two strands.
(d) The enzyme cuts the sugar-phosphate backbone at specific sites on each strand. *(NEET 2019)*

18. A selectable marker is used to
(a) help in eliminating the non-transformants, so that the transformants can be regenerated
(b) identify the gene for a desired trait in an alien organism
(c) select a suitable vector for transformation in a specific crop
(d) mark a gene on a chromosome for isolation using restriction enzyme. *(Odisha NEET 2019)*

19. 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 visualized directly by exposing UV radiation.
Choose correct answer from the options given below.
(a) (i), (iii) and (iv) (b) (i), (ii) and (iii)
(c) (ii), (iii) and (iv) (d) (i), (ii) and (iv)
(Odisha NEET 2019)

20. Which of the following is commonly used as a vector for introducing a DNA fragment in human lymphocytes?
(a) Retrovirus (b) Ti plasmid
(c) λ phage (d) pBR322 *(NEET 2018)*

21. 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)

22. DNA fragments are
(a) negatively charged (b) neutral
(c) either positively or negatively charged depending on their size
(d) positively charged. *(NEET 2017)*

23. A gene whose expression helps to identify transformed cell is known as
(a) vector (b) plasmid
(c) structural gene (d) selectable marker.
(NEET 2017)

24. 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)*

25. A foreign DNA and plasmid cut by the same restriction endonuclease can be joined to form a recombinant plasmid using
(a) EcoRI (b) Taq polymerase
(c) polymerase III (d) ligase. *(NEET-II 2016)*

26. Which of the following restriction enzymes produces blunt ends?
(a) *Sal*I (b) *Eco*RV (c) *Xho*I (d) *Hin*dIII
(NEET-II 2016)

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

28. Which of the following is a restriction endonuclease?
(a) DNase I (b) RNase
(c) *Hind* II (d) Protease
(NEET-I 2016)

29. The introduction of T-DNA into plants involves
(a) exposing the plants to cold for a brief period
(b) allowing the plant roots to stand in water
(c) infection of the plant by *Agrobacterium tumefaciens*
(d) altering the pH of the soil, then heat-shocking the plants. *(2015)*

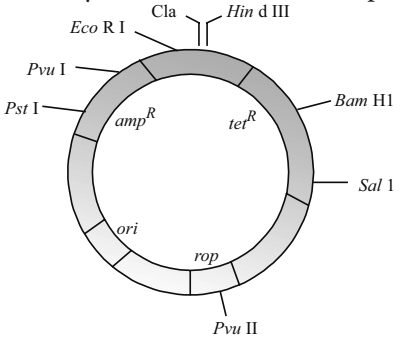
30. Which vector can clone only a small fragment of DNA?
(a) Bacterial artificial chromosome
(b) Yeast artificial chromosome
(c) Plasmid (d) Cosmid *(2014)*

31. Commonly used vectors for human genome sequencing are
(a) T - DNA (b) BAC and YAC
(c) expression vectors (d) T/A cloning vectors. *(2014)*

32. The colonies of recombinant bacteria appear white in contrast to blue colonies of non-recombinant bacteria because of
(a) insertional inactivation of alpha galactosidase in recombinant bacteria
(b) inactivation of glycosidase enzyme in recombinant bacteria
(c) non-recombinant bacteria containing beta galactosidase
(d) insertional inactivation of alpha galactosidase in non-recombinant bacteria. *(NEET 2013)*

33. DNA fragments generated by the restriction endonucleases in a chemical reaction can be separated by
(a) electrophoresis (b) restriction mapping
(c) centrifugation
(d) polymerase chain reaction. *(NEET 2013)*

34. The given figure is the diagrammatic representation of the *E. coli* vector pBR322. Which one of the given options correctly identifies its certain component(s)?



(a) *ori*-original restriction enzyme
(b) *rop*-reduced osmotic pressure
(c) *Hin*dIII, *Eco*RI - selectable markers
(d) amp^R, tet^R–antibiotic resistance genes *(2012)*

35. A single strand of nucleic acid tagged with a radioactive molecule is called
(a) vector (b) selectable marker
(c) plasmid (d) probe. *(2012)*

36. For transformation, micro-particles coated with DNA to be bombarded with gene gun are made up of
(a) silver or platinum (b) platinum or zinc
(c) silicon or platinum (d) gold or tungsten. *(2012)*

37. Biolistics (gene-gun) is suitable for
(a) disarming pathogen vectors
(b) transformation of plant cells
(c) constructing recombinant DNA by joining with vectors
(d) DNA fingerprinting. *(Mains 2012)*

38. In genetic engineering, the antibiotics are used
(a) as selectable markers
(b) to select healthy vectors
(c) as sequences from where replication starts
(d) to keep the cultures free of infection. *(Mains 2012)*

39. Which one of the following represents a palindromic sequence in DNA?
(a) 5′ - GAATTC - 3′ (b) 5′ - CCAATG - 3′
3′ - CTTAAG - 5′ 3′ - GAATCC - 5′
(c) 5′ - CATTAG - 3′ (d) 5′ - GATACC - 3′
3′ - GATAAC - 5′ 3′ - CCTAAG - 5′ *(Mains 2012)*

40. Given below is a sample of a portion of DNA strand giving the base sequence on the opposite strands. What is so special shown in it?
5′ _____ GAATTC _____ 3′
3′ _____ CTTAAG _____ 5′
(a) Replication completed
(b) Deletion mutation
(c) Start codon at the 5′ end
(d) Palindromic sequence of base pairs *(2011)*

41. There is a restriction endonuclease called *Eco*RI. What does "co" part in it stand for?
(a) colon (b) coelom
(c) coenzyme (d) coli *(2011)*

42. Agarose extracted from sea weeds is used in
(a) spectrophotometry (b) tissue culture
(c) PCR (d) gel electrophoresis. *(2011)*

43. 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′ —— GAATTC —— 3′
3′ —— CTTAAG —— 5′
(d) 5′ —— CACGTA —— 3′
3′ —— CTCAGT —— 5′ *(2010)*

44. 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 *(2010)*

45. DNA or RNA segment tagged with a radioactive molecule is called
(a) vector (b) probe
(c) clone (d) plasmid. *(2010)*

46. 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. *(2010)*

47. 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) Bacterium (ii) Plasmid
(iii) Plasmodium (iv) Bacteriophage
(a) (i), (ii) and (iv) (b) (i) only
(c) (i) and (iii) (d) (ii) and (iv)
(Mains 2010)

48. Polyethylene glycol method is used for
(a) biodiesel production
(b) seedless fruit production
(c) energy production from sewage
(d) gene transfer without a vector. *(2009)*

49. Which one of the following is commonly used in transfer of foreign DNA into crop plants?
(a) *Meloidogyne incognita*
(b) *Agrobacterium tumefaciens*
(c) *Penicillium expansum*
(d) *Trichoderma harzianum* *(2009)*

50. Gel electrophoresis is used for
(a) construction of recombinant DNA by joining with cloning vectors
(b) isolation of DNA molecules
(c) cutting of DNA into fragments
(d) separation of DNA fragments according to their size. *(2008)*

51. The linking of antibiotic resistance gene with the plasmid vector became possible with
(a) DNA polymerase (b) exonucleases
(c) DNA ligase (d) endonucleases. *(2008)*

52. Restriction endonuclease
(a) synthesizes DNA
(b) cuts the DNA molecule randomly
(c) cuts the DNA molecule at specific sites
(d) restricts the synthesis of DNA inside the nucleus. *(2006)*

53. Two microbes found to be very useful in genetic engineering are
(a) crown gall bacterium and *Caenorhabditis elegans*
(b) *Escherichia coli* and *Agrobacterium tumefaciens*
(c) *Vibrio cholerae* and a tailed bacteriophage
(d) *Diplococcus sp*. and *Pseudomonas sp*. *(2006)*

54. 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 molecules
(c) are used for *in vitro* DNA synthesis
(d) are synthesized by bacteria as part of their defense mechanism. *(2004)*

55. 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 mm plasmid. *(2004)*

56. 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. *(2004)*

57. Which one of the following bacteria has found extensive use in genetic engineering work in plants ?
(a) *Clostridium septicum*
(b) *Xanthomonas citri*
(c) *Bacillus coagulens*
(d) *Agrobacterium tumefaciens* *(2003)*

58. Which of the following enzymes are used to join bits of DNA?
(a) Ligase (b) Primase
(c) DNA polymerase (d) Endonuclease
(2002)

59. A mutant strain of T_4 - Bacteriophage, R-II, fails to lyse the *E. coli* but when two strains R-IIX and R-IIY are mixed then they lyse the *E. coli*. What may be the possible reason?
(a) Bacteriophage transforms in wild.
(b) It is not mutated.
(c) Both strains have similar cistrons.
(d) Both strains have different cistrons.
(2001)

60. Which of the following cut the DNA from specific places?
(a) *E.*coli restriction endonuclease I
(b) Ligase
(c) Exonuclease
(d) Alkaline phosphate *(2001)*

61. 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. *(2001)*

62. Plasmid has been used as vector because
(a) it is circular DNA which have capacity to join to eukaryotic DNA
(b) it can move between prokaryotic and eukaryotic cells
(c) both ends show replication
(d) it has antibiotic resistance gene. *(2000)*

63. The process of replication in plasmid DNA, other than initiation, is controlled by
(a) mitochondrial gene
(b) plasmid gene
(c) bacterial gene
(d) none of these. *(1999)*

64. Recombinant DNA is achieved by cleaving the pro-DNAs by
(a) ligase
(b) restriction endonuclease
(c) primase
(d) exonucleases. *(1998)*

65. 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.* *(1998)*

66. Restriction endonucleases are
(a) used for *in vitro* DNA synthesis
(b) used in genetic engineering
(c) synthesized by bacteria
(d) present in mammalian cells for degradation of DNA. *(1998)*

67. The restriction enzymes are used in genetic engineering, because
(a) they can cut DNA at specific base sequence
(b) they are nucleases that cut DNA at variable sites
(c) they can degrade harmful proteins
(d) they can join different DNA fragments. *(1995)*

11.3 Processes of Recombinant DNA Technology

68. Match the organism with its use in biotechnology.

(A) *Bacillus thuringiensis*	(i) Cloning vector
(B) *Thermus aquaticus*	(ii) Construction of first *r*DNA molecule
(C) *Agrobacterium tumefaciens*	(iii) DNA polymerase
(D) *Salmonella typhimurium*	(iv) Cry proteins

Select the correct option from the following.

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

(NEET 2020)

69. DNA precipitation out of a mixture of biomolecules can be achieved by treatment with
(a) chilled chloroform
(b) isopropanol
(c) chilled ethanol
(d) methanol at room temperature. *(NEET 2019)*

70. Which one of the following equipments is essentially required for growing microbes on a large scale, for industrial production of enzymes?
(a) Bioreactor (b) BOD incubator
(c) Sludge digester (d) Industrial oven
(NEET 2019)

71. The correct order of steps in Polymerase Chain Reaction (PCR) is
(a) extension, denaturation, annealing
(b) annealing, extension, denaturation
(c) denaturation, extension, annealing
(d) denaturation, annealing, extension.
(NEET 2018)

72. The process of separation and purification of expressed protein before marketing is called
(a) downstream processing
(b) bioprocessing
(c) postproduction processing
(d) upstream processing. *(NEET 2017)*

73. Stirred-tank bioreactors have been designed for
(a) purification of product
(b) addition of preservatives to the product
(c) availability of oxygen throughout the process
(d) ensuring anaerobic conditions in the culture vessel. *(NEET-II 2016)*

74. Which of the following is not a component of downstream processing?
(a) Separation (b) Purification
(c) Preservation (d) Expression
(NEET-II 2016)

75. The *Taq* polymerase enzyme is obtained from
(a) *Bacillus subtilis*
(b) *Pseudomonas putida*
(c) *Thermus aquaticus*
(d) *Thiobacillus ferroxidans*. *(NEET-I 2016)*

76. An analysis of chromosomal DNA using the Southern hybridization technique does not use
(a) electrophoresis (b) blotting
(c) autoradiography (d) PCR. *(2014)*

77. *In vitro* clonal propagation in plants is characterized by
(a) PCR and RAPD
(b) Northern blotting
(c) electrophoresis and HPLC
(d) microscopy. *(2014)*

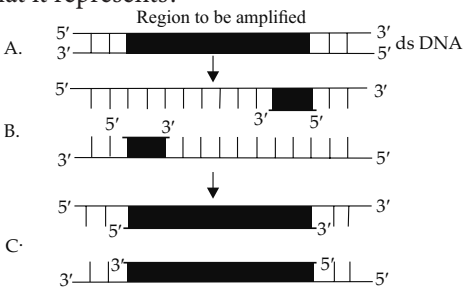
78. Which of the following is not correctly matched for the organism and its cell wall degrading enzyme?
(a) Algae – Methylase
(b) Fungi – Chitinase
(c) Bacteria – Lysozyme
(d) Plant cells – Cellulase *(NEET 2013)*

79. 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.
(Karnataka NEET 2013)

80. PCR and restriction fragment length polymorphism are the methods for
(a) study of enzymes
(b) genetic transformation
(c) DNA sequencing
(d) genetic fingerprinting. *(2012)*

81. 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. *(2012)*

82. The figure below shows three steps (A, B, C) of Polymerase Chain Reaction (PCR). Select the option giving correct identification together with what it represents?



(a) B - denaturation at a temperature of about 98°C separating the two DNA strands
(b) A - denaturation at a temperature of about 50°C
(c) C - extension in the presence of heat stable DNA polymerase
(d) A - annealing with two sets of primers
(Mains 2012)

83. Stirred-tank bioreactors have been designed for
(a) addition of preservatives to the product
(b) purification of the product
(c) ensuring anaerobic conditions in the culture vessel
(d) availability of oxygen throughout the process.
(2010)

ANSWER KEY

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

Hints & Explanations

1. (d) : Vector is a DNA molecule that carries a foreign DNA segment and replicates inside a host cell. The vector DNA and foreign DNA carrying gene of interest are cut by the same restriction endonuclease enzyme to produce complementary sticky ends. With the help of DNA ligase enzyme, the complementary sticky ends of the two DNAs are joined to produce a recombinant DNA (rDNA), which is then introduced into the host cell.

2. (c) : Restriction enzymes recognise specific base sequences in a DNA molecule and cut its strands, *e.g.*, *Eco*RI cuts DNA strands in the base sequence GAATTC.

3. (b) : Vectors are DNA molecules that can carry a foreign DNA segment and replicate inside the host cell. They are used in recombinant DNA technology.

4. (a)

5. (d) : Plasmid is a small circular double stranded DNA molecule present in the cytoplasm of the bacterial cell. It can replicate independently of bacterial chromosome. Due to this characteristic of plasmid, it is used as the vector (vectors are for the transferring of a piece of DNA to target gene) in gene cloning.

6. (a) : DNA restriction endonuclease are important, which cut double-stranded DNA molecules only at sites characterized by a specific nucleotide sequence. Restriction enzymes are isolated from bacterial cells and are tools for molecular biologists.

7. (a) **8. (d)**

9. (b)

10. (b) : Genetic engineering is an experimental manipulation of genetic material, especially for industrial or medical uses. It encompasses the techniques of gene cloning, the DNA modification by changes in sequence arrangement or deletion, and the introduction of novel genes into cells and organisms. It may prove possible to advantageously modify the genes of farmed animals, to correct genetic deficiencies of the human by inserting novel genes. This can be done by breakage of a DNA molecule at two desired places into another DNA molecule of the desired animal.

11. (b) : Plasmids are extrachromosomal genetic element found in many bacteria and in a few eukaryotic cells. Plasmids are closed circles of double-stranded DNA ranging in size from 1 to 200 kilobases. They frequently carry genes conferring antibiotic resistance. Plasmids are becoming important tools for genetic engineering since they have the ability to replicate and migrate to daughter cells. Plasmids are widely used as carriers of cloned genes, for example the *E. coli* plasmid pBR322. When plasmids are used as cloning vectors and carry a novel DNA sequence they are referred to as chimeric plasmids.

12. (d) **13. (a)**

14. (a) : The palindromes in DNA are base pair sequences that are same when read forward (left to right) or backward (right to left) from a central axis of symmetry.

Thus, $\frac{\text{GAATTC}}{\text{CTTAAG}}$ is a palindromic sequence which is recognised by *ECo*RI.

15. (b) : Ori site 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 also responsible for controlling the copy number of the linked DNA in the vector. So, if one wants to recover many copies of the target DNA it should be cloned in a vector whose origin support high copy number.

16. (b) : In gel electrophoresis , separated DNA fragments can be visualised only after staining the DNA with a compound *i.e.*, ethidium bromide and followed by exposure to UV radiation as bright orange coloured bands.

17. (c) : The restriction endonuclease enzyme inspects the length of a DNA sequence. Once it recognises specific sequence, it binds to the DNA and cuts each of the two strands of the double helix at specific points in their sugar phosphate backbone. Special sequence in the DNA recognised by restriction endonuclease is called palindromic nucleotide sequence.

18. (a)

19. (d) : DNA is a negatively charged molecule, so they can be separated by forcing them to move towards the anode under an electric field. DNA fragments separate according to size through the pores of agarose gel. The separated DNA fragments can be visualised only after staining the DNA with a compound known as ethidium bromide then followed by exposure to UV radiation.

20. (a) : Retroviruses cause cancer in animals including humans. So modified retroviruses are used to transfer desirable genes into animal cells. It is used in gene therapy, in which lymphocytes from blood of patient are taken and grown in culture medium outside the body, a functional gene is introduced by using a retroviral vector into these lymphocytes which are again reintroduced into the patient body.

21. (c) : The separated DNA fragments can be seen only after staining them with a compound known as ethidium bromide (EtBr) followed by exposure to UV radiation as bright orange coloured bands.

22. (a)

23. (d) : Some genes called "selectable markers" help in selecting those host cells which contain the vectors (transformants) and eliminating the non-transformants.

24. (a) : Electrophoresis is a technique used for the separation of substances of different ionic properties. Since the DNA fragments are negatively charged molecules, they can be separated by allowing them to move towards the anode. DNA fragments move towards the anode according to their molecule size through the pores of agarose gel. Thus, the smaller fragments move farther away as compared to larger fragments.

25. (d) : Ligase is a class of enzymes that catalyse the formation of covalent bonds using the energy released by the cleavage of ATP. Ligases are important in the synthesis and repair of many biological molecules, including DNA ligase and used in genetic engineering to insert foreign DNA into cloning vectors.

26. (b) : *Eco*RV is a type II restriction endonuclease isolated from certain strains of *E.coli*. It creates blunt ends. It recognises the palindromic sequence of 6 bases. *Sal*I, *Xho*I and *Hind*III restriction enzymes produce sticky ends.

27. (b) : Plasmids are extra-chromosomal, self-replicating, usually circular, double-stranded DNA molecules that serve as vectors which carry foreign DNA segment and replicate inside host cell.

28. (c) : *Hind* II is the first restriction endonuclease. It was isolated from *Haemophilus influenzae* Rd. It always cut DNA at specific position producing blunt ends. DNase I is an endonuclease that cleaves DNA preferentially at phosphodiester linkages adjacent to a pyrimidine nucleotide. RNase is a type of nuclease that catalyses the degradation of RNA into smaller components. It can be endoribonuclease or exoribonuclease. A protease is an enzyme that perform proteolysis, *i.e.*, protein catabolism by hydrolysis of the peptide bonds.

29. (c) : Ti plasmid (tumor inducing) from the soil bacterium *Agrobacterium tumefaciens* is effectively used as vector for gene transfer to plant cells. The part of Ti plasmid transferred into plant cell DNA, is called the T-DNA. This T-DNA with desired DNA spliced into it, is inserted into the chromosomes of the host plant where it produces copies of itself. Such plant cells are then cultured, induced to multiply and differentiate to form plantlets. By transferring into soil, the plantlets grow into mature plants, carrying the foreign gene, expressed throughout the new plant.

30. (c) : Plasmids have been modified to be used as vectors. They can clone DNA fragments of about 10 kbp size while cosmid can carry upto 45 kbp, YAC can carry upto 1000-2500 kbp and BAC can carry around 300 – 350 Kbp long DNA fragments.

31. (b) : Bacterial artificial chromosome (BAC) vectors are based on natural, extra-chromosomal plasmid of *E. coli*. BAC vector contains genes for replication and maintenance of the F-factor, a selectable marker and cloning site. These vectors can accommodate upto 300-350 kb of foreign DNA and are also being used in genome sequencing project. Yeast artificial chromosome (YAC) vectors are used to clone DNA fragments of more than 1Mb in size. Therefore, they have been exploited extensively in mapping the large genomes, *e.g.*, in the Human Genome Project. These vectors contain the telomeric sequence, the centromere and the autonomously replicating sequence from yeast chromosomes.

32. (c) : The presence of restriction sites within the markers tet^r and amp^r of plasmid permits an easy selection for cells transformed with recombinant plasmid. Insertion of the DNA fragment into the plasmid makes antibiotic resistance genes nonfunctional, for example, insertion of the DNA fragment into the plasmid (pBR322) using *Pst* I or *Pvu* I makes amp^r nonfunctional. Bacterial cells containing such a recombinant pBR322 will be unable to grow in the presence of ampicillin, but will grow on tetracycline. This process, however, becomes burdensome because it requires simultaneous plating on two plates having different antibiotics. Thus, alternative selectable marker is developed to differentiate recombinants and non-recombinants on the basis of their ability to produce colour in the presence of a chromogenic substance. Here, a recombinant DNA is inserted in the coding sequence of an enzyme β-galactosidase. pUC 18 plasmid contains this gene which allows it to produce β-galactosidase which degrades certain sugars and produces a blue pigment when exposed to specific substrate analog. If the plasmid in the bacterium does not have an insert, *i.e.*, is non-recombinant, the presence of chromogenic substrate gives blue coloured colonies. Presence of insert in the plasmid in recombinant bacterium does not produce any colour, such bacterial colonies are marked as recombinant colonies.

33. (a)

34. (d) : In pBR322, *ori*-represents site or origin of replication, *rop*-codes for proteins that take part in the replication of plasmid. *Hind* III, *Eco*RI- recognition sites of restriction endonucleases. amp^R and tet^R - antibiotic resistance genes.

35. (d) : Probes are single stranded, radiolabelled molecules of nucleic acids with known sequence. The

probes having sequence complementary to the gene to be identified are supplied. They bind with the particular gene segment. Radiation imaging identifies the location of that particular segment which bind with probe. Probes are used as identification tool.

36. (d) : A gene or a biolistic particle delivery system, originally designed for plant transformation, is a device for injecting cells with genetic information. The payload is an elemental particle of a heavy metal such as gold or tungsten coated with plasmid DNA. The device is used to transform almost any type of cell including plants, and is not limited to genetic material of the nucleus. It can also transform organelles, including plastids.

37. (b) : Biolistics is a technique for introducing genetic material into living cells, especially plant cells, in which DNA-coated microscopic particles (tungsten or gold particles) are bombarded with a very high velocity into the target cell using a special gun. The microprojectiles, typically 1mm in diameter, are accelerated to high velocity by a specially modified small calibre gun and penetrate the cell walls and plasma membrane with minimal damage. Hence, the novel DNA can be inserted into intact plant cells ultimately transforming it without using a vector.

38. (a) : Selectable markers are those genes which help in selecting those host cells which contain vectors (*i.e.*, transformants) and eliminating the non-transformants. The genes encoding resistance to antibiotics such as tetracycline, ampicillin, kanamycin, etc., are useful selectable markers for *E.coli*. Plasmid pBR322 has two resistance genes – ampicillin resistance (amp^r) and tetracycline resistance (tet^r) which are considered useful for selectable markers. The presence of restriction sites within the markers tet^r and amp^r permits an easy selection for cells transformed with the recombinant pBR322. Insertion of the DNA fragment into the plasmid using enzyme *Pst* I or *Pvu* I places the DNA insert within the gene amp^r; this makes amp^r nonfunctional. Bacterial cells containing such a recombinant pBR322 will be unable to grow in the presence of ampicillin, but will grow on tetracycline. Similarly, when restriction enzyme *Bam* HI or *Sal I* is used, the DNA insert is placed within the gene tet^rmaking it nonfunctional. Bacterial cells possessing such a recombinant pBR322 will, therefore, grow on ampicillin but not on tetracycline.

39. (a) : Palindromes are groups of letters that form the same words when read both forward and backward, *e.g.*, "MALAYALAM". As against a word-palindrome where the same word is read in both directions, the palindrome in DNA is a sequence of base pairs that reads same on the two strands when orientation of reading is kept the same. For example, the following sequences read the same on the two strands in $5' \rightarrow 3'$ direction. This is also true if read in the $3' \rightarrow 5'$ direction. In this case, it is

$5'$ — GAATTC — $3'$

$3'$ — CTTAAG — $5'$.

40. (d)

41. (d) : The enzyme restriction endonuclease *Eco*RI is found in the colon bacteria *E. coli*. So, here 'co' stands for *coli*. According to nomenclature of restriction enzyme, the first letter used for the enzyme is the first letter of the genus name (in italics) of the bacterium, then comes the first two letters of its species (also in italics), next is the strain of the organism. At last is a Roman numeral signifying the order of discovery. Here, the enzyme *Eco*RI was isolated from the bacterium *Escherichia coli* (co), strain RY13(R) and it was first endonuclease (I) isolated from *E.coli*.

42. (d) : In gel electrophoresis, DNA fragments separate (resolve) according to their size through sieving effect provided by the agarose gel. Agarose is a natural polymer extracted from sea weeds and is commonly used as a matrix.

43. (c) : Palindromic nucleotide sequences in the DNA molecule are groups of bases that form the same sequence when read in both forward and backward direction. In the given question, only option (c) represents a palindromic sequence, that can be easily cut at about the middle by some particular restriction enzyme.

44. (d) : Retroviruses in animals have the ability to transform normal cells into cancerous cells. We have transformed these pathogens into useful vectors for delivering genes of interest to humans. Retroviruses have been disarmed and are now used to deliver desirable genes into animal cells. So, once a gene or a DNA fragment has been ligated into a suitable retroviral vector it is transferred into a bacterial, plant or animal host (where it multiplies).

45. (b) : *Refer to answer 35.*

46. (a) : Restriction endonucleases were found by Arber in 1962 in bacteria. They act as "molecular scissors" or chemical scalpels. They recognize the specific base sequence at palindrome sites in DNA duplex and cut its strands. For example, restriction endonuclease *Eco*RI found in the colon bacteria *E. coli* recognizes the base sequence GAATTC in DNA duplex and cuts its strands between G and A.

47. (d) : Plasmid and bacteriophage are used as vectors in genetic engineering. Plasmid is an autonomously replicating circular extra-chromosomal DNA found in

bacteria. They can be transferred from cell to cell in a bacterial colony. This characteristic is being used in biotechnology for transferring desirable gene into target gene of the host. Bacteriophage is a bacterial virus which can infect it, quickly multiply within and destroy (lyse) their host (virus) cells. During infection bacteriophages inject their DNA into these cells. The injected DNA selectively replicate and are expressed in the host that results in a multiplication of phages that ultimately burst out of the cell (by lysis). This ability of transferring DNA from the phage genome to specific host during infection process gave scientists the idea that specially designed phage vectors could be used for gene cloning.

48. (d) : Direct gene transfer is the transfer of naked DNA into plant cells, but the presence of rigid plant cell wall acts as a barrier to uptake. Therefore, protoplasts are the favoured target for direct gene transfer. Polyethylene glycol mediated DNA uptake is a direct gene transfer method that utilizes the interaction between PEG, naked DNA, salts and the protoplast membrane to effect transport of the DNA into the cytoplasm.

49. (b) : *Agrobacterium tumefaciens* has been extensively used in genetic engineering experiments. It is the causative agent of crown gall, an important disease of many commercial crops. This disease has come to be recognized in recent years as being caused by a DNA plasmid (Ti plasmid) carried by bacterium and transferred to the plant cells. Following the discovery of the relationship between crown gall and the Ti plasmid, this plasmid has come to be widely used in plant genetic engineering as a vector in order to inject a novel gene in host plant to form a transgenic plant.

50. (d) : Electrophoresis is a technique used for the separation of substances of different ionic properties. Since the DNA fragments are negatively charged molecules, they can be separated by allowing them to move towards the anode. DNA fragments move towards the anode according to their molecule size through the pores of agarose gel. Thus, the smaller fragments move farther away as compared to larger fragments.

51. (c) : The construction of the first recombinant DNA emerged from the possibility of linking a gene encoding antibiotic resistance with a native plasmid. 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. This plasmid DNA acts as vector to transfer the piece of DNA attached to it. 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.

52. (c)

53. (b) : *E.coli* contains many important standard cloning vectors widely used in gene cloning experiments like pBR322 contains origin of replication (*ori*). Other cloning vectors like PACYC177, pBR324, contain ampicillin resistance gene are also found in *E.coli*. Among higher plants, Ti plasmid of *Agrobacterium tumefaciens* and Ri plasmid of *A. rhizogenes* is the best known vector. T-DNA from Ti or Ri plasmid of *Agrobacterium* is considered to be a very potential vector for cloning experiments with higher plants.

54. (d) : Restriction endonucleasses are enzymes that digest double stranded DNA following recognition of specific nucleotide sequences. This is achieved by cleaving the two phosphodiester bonds, one within each strand of the DNA duplex. They are found in bacteria and their function in bacteria is to cut up any invading virus as a part of its defense mechanism, thus restricting the multiplication of viruses in the bacterial cell. Different species of bacteria produce different restriction endonucleases.

55. (c) : *Refer to answer 29.*

56. (b) : *Agrobacterium tumefaciens* is the causative agent of crown gall, an important disease of many commercial crops. This disease has come to be recognized in recent years as being caused by a DNA plasmid (Ti plasmid) carried by bacterium and transferred to the plant cells.

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58. (a) : Ligases are used to join bits of DNA. Primase is an RNA polymerase, used to initiate DNA synthesis. DNA polymerase enzyme catalyses the synthesis of DNA. Endonuclease, causes the splicing of the intron carrying the coding sequence of the same endonuclease.

59. (d) : A mutant strain of T_4-bacteriophage, RII, fails to lyse the *E.coli* but when two strains R-IIX and R-IIY are mixed then they lyse the *E.coli* because both strains have different cistrons.

60. (a)

61. (b) : A plasmid is a DNA molecule separate from the chromosomal DNA and capable of autonomous replication. In many cases, it is typically circular and double-stranded. 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.

62. (a)

63. (c) : The DNA plasmid replicates in a semi-conservative manner. The initiation of replication is controlled by plasmid gene and elongation and termination are controlled by bacterial genes.

64. (b) : Recombinant DNA is the product obtained after isolating a specific DNA segment and then inserting it into another DNA molecule at a desired position. Restriction endonucleases are the enzymes that digest DNA at specific sites to isolate a specific DNA segment. Thus they are required for producing recombinant DNA.

65. (d) : *Refer to answer 53.*

66. (b)

67. (a) : DNA restriction endonuclease cut double-stranded DNA molecules only at sites characterized by a specific nucleotide sequence.

68. (b)

69. (c) : In order to cut the DNA with restriction enzymes, it needs to be in pure form, free from other macromolecules. Since the DNA is enclosed by the membranes, we have to break the cell open to release DNA and other macromolecules like RNA, proteins, polysaccharides and lipids. It is obtained by treating the bacterial cells/plant or animal tissue with enzymes. Other molecules are removed by appropriate treatments and purified DNA ultimately precipitates out after the addition of chilled ethanol.

70. (a)

71. (d) : A single PCR amplification cycle involves three basic steps, denaturation heating of target DNA to high temperature resulting in separation of two strands, annealing two oligonucleotide primers anneal or hybridise to each single template DNA and extension. *Taq* DNA polymerase synthesises DNA between primers and primers extend towards each other such that DNA stranded segment lying between the two is copied.

72. (a) : After the formation of the product in the bioreactor it undergoes some processes before a finished product is ready for marketing. The process includes separation and purification of products which are collectively called downstream processing.

73. (c) : A stirred-tank reactor is usually cylindrical or with a curved base to facilitate the mixing of the reactor contents. The stirrer facilitates, even mixing and oxygen availability throughout the bioreactor.

74. (d) : After the formation of the product in bioreactor, it undergoes some processes before a finished product to be ready for marketing. Downstream processing includes separation and purification process. The product obtained is subjected to quality control, testing and kept in suitable preservatives.

75. (c) : *Taq* polymerase, generally used in PCR is isolated from thermophilic bacterium *Thermus aquaticus*.

76. (d) : PCR is used only for amplification of DNA. It is not directly involved in Southern hybridisation technique.

77. (a) : Clonal propagation can be characterized by PCR and RAPD. The polymerase chain reaction (PCR) technique, generates microgram (μg) quantities of DNA copies (upto billion copies) of the desired DNA (or RNA) segment, present even as a single copy in the initial preparation, in a matter of few hours. RAPD stands for Random Amplification of Polymorphic DNA. It is a type of PCR, but the segments of DNA that are amplified are random. No knowledge of the DNA sequence for the targeted gene is required, as the primers will bind somewhere in the sequence, but it is not certain exactly where. Its resolving power is much lower than targeted, species specific DNA comparison methods, such as short tandem repeats.

78. (a) : Cell wall of algae is made up of cellulose, pectin and mucilage. These substances cannot be degraded by methylase. Methylase is a type of transferase enzyme that transfers a methyl group from a donor to an acceptor.

79. (a) : Ethanol is much less polar than water. Adding it to the solution disrupts the screening charges exerted by water. The electrical attraction between phosphate and any positive ions (Na^+) present in solution becomes strong enough to form a stable ionic bond and DNA precipitates. Ethanol precipitation is a widely used technique to purify, or concentrate nucleic acid.

80. (d)

81. (d) : In PCR, *Taq* polymerase is used which is obtained from *Thermus aquaticus* bacteria. It is a relatively thermostable enzyme thus used in PCR as during the process the step involving denaturation of DNA strands requires high temperature.

82. (c)

83. (d) : *Refer to answer 73.*

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