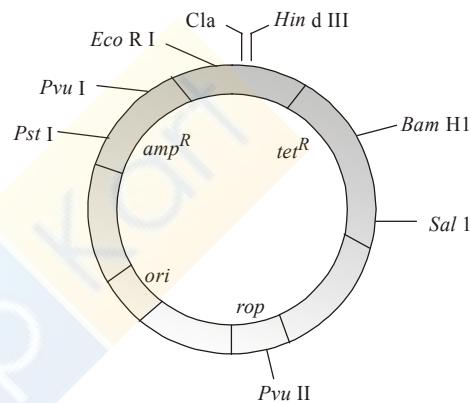


# Chapter 33

## Biotechnology: Principles and Processes

- 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)
- DNA fragments are  
(a) negatively charged  
(b) neutral  
(c) either positively or negatively charged depending on their size  
(d) positively charged. (NEET 2017)
- A gene whose expression helps to identify transformed cell is known as  
(a) vector (b) plasmid  
(c) structural gene  
(d) selectable marker. (NEET 2017)
- 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)
- 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)
- 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)
- 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)
- Which of the following is not a component of downstream processing?  
(a) Separation (b) Purification  
(c) Preservation (d) Expression (NEET-II 2016)
- Which of the following restriction enzymes produces blunt ends?  
(a) *SalI* (b) *EcoRV*  
(c) *XhoI* (d) *HindIII* (NEET-II 2016)
- 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)
- The *Taq* polymerase enzyme is obtained from  
(a) *Bacillus subtilis*  
(b) *Pseudomonas putida*  
(c) *Thermus aquaticus*  
(d) *Thiobacillus ferrooxidans*. (NEET-I 2016)
- Which of the following is a restriction endonuclease?  
(a) DNase I (b) RNase  
(c) *Hind II* (d) Protease (NEET-I 2016)
- Which of the following is not required for any of the techniques of DNA fingerprinting available at present?  
(a) Restriction enzymes  
(b) DNA-DNA hybridisation  
(c) Polymerase chain reaction  
(d) Zinc finger analysis (NEET-I 2016)
- The DNA molecule to which the gene of interest is integrated for cloning is called

- (a) template (b) carrier  
(c) transformer (d) vector. (2015)
15. The cutting of DNA at specific locations became possible with the discovery of  
(a) selectable markers (b) ligases  
(c) restriction enzymes  
(d) probes. (2015)
16. An analysis of chromosomal DNA using the Southern hybridization technique does not use  
(a) electrophoresis (b) blotting  
(c) autoradiography (d) PCR. (2014)
17. Which vector can clone only a small fragment of DNA?  
(a) Bacterial artificial chromosome  
(b) Yeast artificial chromosome  
(c) Plasmid  
(d) Cosmid (2014)
18. Commonly used vectors for human genome sequencing are  
(a) T-DNA  
(b) BAC and YAC  
(c) expression vectors  
(d) T/A cloning vectors. (2014)
19. 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)
20. 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)
21. 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)
22. 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)
23. 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) *HindIII*, *EcoRI* - selectable markers  
(d) *amp<sup>R</sup>*, *tet<sup>R</sup>*-antibiotic resistance genes (2012)
24. PCR and restriction fragment length polymorphism are the methods for  
(a) study of enzymes  
(b) genetic transformation  
(c) DNA sequencing  
(d) genetic fingerprinting. (2012)
25. A single strand of nucleic acid tagged with a radioactive molecule is called  
(a) vector  
(b) selectable marker  
(c) plasmid  
(d) probe. (2012)
26. 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)

27. For transformation, micro-particles coated with DNA to be bombarded with gene gun are made up of
- silver or platinum
  - platinum or zinc
  - silicon or platinum
  - gold or tungsten.

(2012)

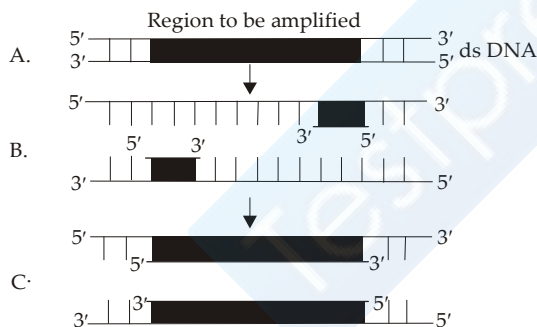
28. Biolistics (gene-gun) is suitable for
- disarming pathogen vectors
  - transformation of plant cells
  - constructing recombinant DNA by joining with vectors
  - DNA fingerprinting.

(Mains 2012)

29. In genetic engineering, the antibiotics are used
- as selectable markers
  - to select healthy vectors
  - as sequences from where replication starts
  - to keep the cultures free of infection.

(Mains 2012)

30. 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?



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

(Mains 2012)

31. Which one of the following represents a palindromic sequence in DNA?
- 5' - GAATTC - 3'    (b) 5' - CCAATG - 3'  
3' - CTTAAG - 5'    3' - GAATCC - 5'
  - 5' - CATTAG - 3'    (d) 5' - GATACC - 3'  
3' - GATAAC - 5'    3' - CCTAAG - 5'

(Mains 2012)

32. 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?



- Replication completed
- Deletion mutation
- Start codon at the 5' end
- Palindromic sequence of base pairs (2011)

33. There is a restriction endonuclease called *EcoRI*. What does "co" part in it stand for?

- colon                      (b) coelom
- coenzyme                (d) *coli* (2011)

34. Agarose extracted from sea weeds is used in

- spectrophotometry
- tissue culture
- PCR
- gel electrophoresis. (2011)

35. Which one of the following techniques made it possible to genetically engineer living organisms?

- Recombinant DNA techniques
- X-ray diffraction
- Heavier isotope labelling
- Hybridization (Mains 2011)

36. Which one of the following is used as vector for cloning genes into higher organisms?

- Baculovirus
- Salmonella typhimurium*
- Rhizopus nigricans*
- Retrovirus (2010)

37. DNA or RNA segment tagged with a radioactive molecule is called

- vector                      (b) probe
- clone                      (d) plasmid. (2010)

38. Restriction endonucleases are enzymes which
- make cuts at specific positions within the DNA molecule
  - recognize a specific nucleotide sequence for binding of DNA ligase
  - restrict the action of the enzyme DNA polymerase
  - remove nucleotides from the ends of the DNA molecule. (2010)

39. Stirred-tank bioreactors have been designed for

- addition of preservatives to the product
- purification of the product
- ensuring anaerobic conditions in the culture vessel
- availability of oxygen throughout the process. (2010)

40. Which of the following are used in gene cloning?  
 (a) Nucleoids (b) Lomasomes  
 (c) Mesosomes (d) Plasmids (2010)
41. 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) & (iv) (b) (i) only  
 (c) (i) & (iii) (d) (ii) & (iv)  
 (Mains 2010)
42. 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)
43. 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)
44. The linking of antibiotic resistance gene with the plasmid vector became possible with  
 (a) DNA polymerase (b) exonucleases  
 (c) DNA ligase (d) endonucleases.  
 (2008)
45. 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)
46. 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)
47. 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)
48. In transgenics, expression of transgene in target tissue is determined by  
 (a) enhancer (b) transgene  
 (c) promoter (d) reporter. (2004)
49. In recent years, DNA sequences (nucleotide sequence) of *mtDNA* and Y chromosomes were considered for the study of human evolution, because  
 (a) they are small and therefore, easy to study  
 (b) they are uniparental in origin and do not take part in recombination  
 (c) their structure is known in greater detail  
 (d) they can be studied from the samples of fossil remains. (2003)
50. Which one of the following bacteria has found extensive use in genetic engineering work in plants?  
 (a) *Clostridium septicum*  
 (b) *Xanthomonas citri*  
 (c) *Bacillus coagulans*  
 (d) *Agrobacterium tumefaciens* (2003)
51. Manipulation of DNA in genetic engineering became possible due to the discovery of  
 (a) restriction endonuclease  
 (b) DNA ligase (c) transcriptase  
 (d) primase. (2002)
52. A mutant strain of T4 - 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)
53. In Lederberg's replica plating experiment what shall be used to obtain streptomycin resistant strain  
 (a) minimal medium and streptomycin  
 (b) complete medium and streptomycin  
 (c) only minimal medium  
 (d) only complete medium. (2001)
54. Which of the following cut the DNA from specific places?  
 (a) *E.coli* restriction endonuclease I

- (b) Ligase  
(c) Exonuclease  
(d) Alkaline phosphate (2001)
55. 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)
56. The bacteria generally used for genetic engineering is  
(a) *Agrobacterium* (b) *Bacillus*  
(c) *Pseudomonas* (d) *Clostridium*. (2000)
57. 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)
58. 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)
59. Which of the following is related to genetic engineering?  
(a) Heterosis (b) Mutation  
(c) Plasmid (d) Plasmid (1999)
60. Recombinant DNA is achieved by cleaving the pro-DNAs by  
(a) ligase  
(b) restriction endonulcease  
(c) primase  
(d) exonucleases. (1998)
61. Two bacteria found to be very useful in genetic engineering experiments are  
(a) *Nitrobacter* and *Azotobacter*  
(b) *Rhizobium* and *Diplococcus*  
(c) *Nitrosomonas* and *Kliebsiella*  
(d) *Escherichia* and *Agrobacterium*. (1998)
62. 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)
63. 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)
64. 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)
65. Which of the following organelles is related with genetic engineering?  
(a) Mitochondria (b) Plasmids  
(c) Golgi bodies (d) Lysosomes (1994)

Answer Key

1. (c) 2. (a) 3. (d) 4. (a) 5. (a) 6. (c) 7. (d) 8. (d) 9. (b) 10. (b)  
11. (c) 12. (c) 13. (d) 14. (d) 15. (c) 16. (d) 17. (c) 18. (b) 19. (c) 20. (a)  
21. (a) 22. (a) 23. (d) 24. (d) 25. (d) 26. (d) 27. (d) 28. (b) 29. (a) 30. (c)  
31. (a) 32. (d) 33. (d) 34. (d) 35. (a) 36. (d) 37. (b) 38. (a) 39. (d) 40. (d)  
41. (d) 42. (b) 43. (d) 44. (c) 45. (c) 46. (b) 47. (d) 48. (d) 49. (b) 50. (d)  
51. (a) 52. (d) 53. (a) 54. (a) 55. (b) 56. (a) 57. (a) 58. (c) 59. (d) 60. (b)  
61. (d) 62. (b) 63. (b) 64. (a) 65. (b)

## EXPLANATIONS

1. **(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.
2. **(a)**
3. **(d)** : Some genes called “selectable markers” help in selecting those host cells which contain the vectors (transformants) and eliminating the non-transformants.
4. **(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 molecules size through the pores of agarose gel. Thus, the smaller fragments move farther away as compared to larger fragments.
5. **(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.
6. **(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.
7. **(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.
8. **(d)** : After the formation of the product in bioreactor, it undergoes through 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.
9. **(b)** : *EcoRV* 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 as shown here:  
*SalI*, *XhoI* and *HindIII* restriction enzymes produce sticky ends.
10. **(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.
11. **(c)** : *Taq* polymerase, generally used in PCR is isolated from thermophilic bacterium *Thermus aquaticus*.
12. **(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 non-specially. 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.
13. **(d)** : Any small, functional, freely folded domain in which coordination of one or more zinc ions is required to stabilise its structure is known as zinc finger. The zinc finger domains are widely dispersed in eukaryotic genomes and are actively involved in sequence specific binding to DNA/RNA and contribute in protein-protein recognitions.
14. **(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.
15. **(c)** : Restriction enzymes recognise specific base sequences in a DNA molecule and cut its strands, *e.g.*, *EcoRI* cuts DNA strands in the base sequence GAATTC.
16. **(d)** : PCR is used only for amplification of DNA. It is not directly involved in Southern hybridisation technique.
17. **(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.

**18. (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.

**19. (c) :** The presence of restriction sites within the markers *tet<sup>r</sup>* and *amp<sup>r</sup>* 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<sup>r</sup>* 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  $\beta$ -galactosidase. pUC 18 plasmid contains this gene which allows it to produce  $\beta$ -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.

**20. (a) :** Refer to answer 4.

**21. (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.

**22. (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 ( $\text{Na}^+$ ) 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.

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

**24. (d) :** Polymerase chain reaction (PCR) is used to amplify a small DNA fragment to obtain its large quantity. PCR is very helpful in DNA fingerprinting in such cases where the culprit has to be identified from a very small blood, semen or other cell sample from a crime scene.

**25. (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.

**26. (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.

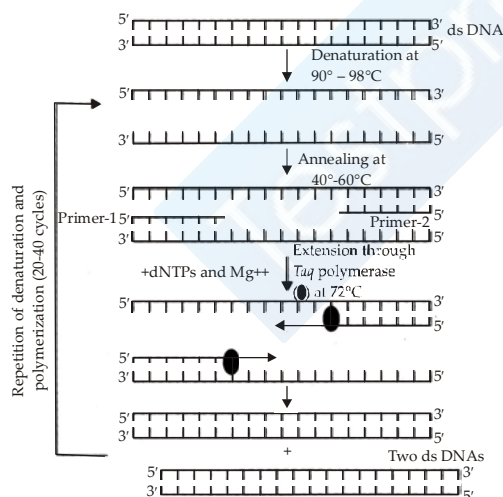
**27. (d) :** A gene or a biolistic particle delivery system, originally designed for plant transformation, is a device for injecting cells with genetic information. The payload is an elemental particle of a heavy metal such as gold or tungsten coated with plasmid DNA. The device is used to transform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

**28. (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.

**29. (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<sup>r</sup>*) and tetracycline resistance (*tet<sup>r</sup>*) which are considered useful for selectable markers.

The presence of restriction sites within the markers *tet<sup>r</sup>* and *amp<sup>r</sup>* 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<sup>r</sup>*; this makes *amp<sup>r</sup>* 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<sup>r</sup>* making it nonfunctional. Bacterial cells possessing such a recombinant pBR322 will, therefore, grow on ampicillin but not on tetracycline.

**30. (c) :** A schematic representation of PCR can be illustrated as follow.



**31. (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′ → 3′ direction.

This is also true if read in the 3′ → 5′ direction. In this case, it is



**32. (d) :** Refer to answer 31.

**33. (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*.

**34. (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.

**35. (a)**

**36. (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).

**37. (b) :** Refer to answer 25.

**38. (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.

**39. (d) :** A stirred-tank bioreactor is usually cylindrical or with a curved base to facilitate the mixing of the reaction contents. The stirrer facilitates even mixing and oxygen availability throughout the bioreactor. Alternatively air can be bubbled through the reactor.

**40. (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.

**41. (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.

**42. (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.

**43. (d) :** Refer to answer 4.

**44. (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.

**45. (c) :** Refer to answer 38.

**46. (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, PRK 64.6 contain ampicillin resistance gene they 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.

**47. (d) :** Restriction endonucleases 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.

**48. (d) :** The plants, in which a functional foreign gene has been incorporated by any biotechnological methods that generally is not present in plant, are called transgenic plants. When plant cell are transformed by any of the transformation methods it is necessary to isolate the transformed cells/tissue. There are certain selectable marker genes present in vectors that facilitate the selection process. In transformed cells the selectable marker genes or are introduced through vector. There is a number of marker genes which are commonly described as reporter genes screenable genes. Some of the reporter genes which are most commonly used in plant transformation are : cat, gus, lux, nptII., etc.

**49. (b) :** Sequence of both *mtDNA* and Y chromosomes are considered for the study of human evolution because they are uniparental in origin. *mtDNA* is inherited along with the maternal cytoplasm and Y chromosome is inherited from father. So they do not take part in recombination. In addition, *mtDNA* has a higher mutation rate than nuclear DNA so that it is more useful for short term evolutionary studies.

**50. (d) :** Refer to answer 42.

**51. (a) :** DNA restriction endonuclease are important class of restriction exonucleases, class

II, 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. Several hundred restriction enzymes are now known, each with a specific sequence requirement dictating where it will cut DNA. Some, such as *Hind* III, make staggered cuts leaving 'sticky ends', three nucleotides long protruding on one strand from each severed terminus; others make clean cuts in both strands at the same place and thus generate 'blunt ends'. Digesting DNA with a restriction enzyme therefore creates a characteristic set of fragments, which can be isolated by electrophoresis and subsequently analysed.

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

**53. (a) :** If streptomycin resistant mutant are to be obtained, material should be allowed to grow on medium lacking streptomycin so that both mutant and wild types may grow. These colonies are imprinted on petriplates to form the master pattern and other plates having streptomycin can then be pressed on velveteen to get an impression. The plate now containing only mutants for streptomycin resistance will grow.

**54. (a)**

**55. (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. The term plasmid was first introduced by the American molecular biologist Joshua Lederberg in 1952.

**56. (a)**

**57. (a) :** Refer to answer 41.

**58. (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.

**59. (d)**

**60. (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.

**61. (d) :** Refer to answer 46.

**62. (b) :** Refer to answer 38.

**63. (b) :** Refer to answer 51.

**64. (a) :** Refer to answer 51.

**65. (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 one 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, as 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.

