CHAPTER
11

Biotechnology : Principles and Processes

CASE STUDY / PASSAGE BASED QUESTIONS

1

Read the following and answer any four questions from 1(i) to 1(v) given below:
Gene manipulation is a fast emerging science. It started with development of recombinant DNA molecule. It is named variously as DNA manipulation biotechnology, recombinant DNA technology and genetic engineering. This technology, that mostly involves cutting and pasting of desired DNA fragments, is based on two important discoveries in bacteria, *i.e.*, presence of plasmid in bacteria and restriction endonucleases. Paul Berg was able to introduce a gene of SV-40 into a bacterium. The science of recombinant DNA technology took birth when Cohen and Boyer (1973) were able to introduce a piece of gene containing foreign DNA into plasmid of *E.coli*.

(i) Biotechnology is also known as
(a) DNA manipulation biotechnology (b) recombinant DNA technology
(c) genetic engineering (d) all of these.

(ii) A bacterial plasmid is a/an
(a) extra chromosomal material that do not replicate
(b) extra chromosomal material that undergo replication with or without chromosomal DNA
(c) tubular structures that help in conjugation
(d) bristle like solid structure that help in adhesion.

(iii) Father of genetic engineering is
(a) Paul Berg (b) Arber (c) Nathan (d) Smith.

(iv) Which of the following is used by Paul Berg to introduce a gene of SV-40 in a bacterium?
(a) *E. coli* (b) cos-plasmids (c) Lambda phage (d) None of these

(v) Read the given statements and select the correct option.
Assertion : Biotechnology started with the development of recombinant DNA molecule.
Reason : Biotechnology mostly involves cutting and pasting of desired DNA fragments.
(a) Both assertion and reason are true and reason is the correct explanation of assertion.

Syllabus
Genetic Engineering (Recombinant DNA Technology).

(b) Both assertion and reason are true but reason is not the correct explanation of assertion.
(c) Assertion is true but reason is false.
(d) Both assertion and reason are false.

2

Read the following and answer any four questions from 2(i) to 2(v) given below:
Restriction endonuclease was isolated for the first time by W. Arber in 1962 in bacteria. Restriction endonucleases cut the DNA duplex at specific points therefore they are also called as molecular scissors or biological scissors. Three types of restriction endonucleases are Type I, Type II and Type III but only Type II restriction endonucleases are used in recombinant DNA technology. Restriction endonuclease Eco*R* I recognises the base sequence GAATTC in DNA duplex and cut strands between G and A.

(i) Only type II restriction enzymes are used in gene manipulation because
(a) ATP is not required for cleaving
(b) it consists of three different subunits
(c) it makes cleavage or cut in both the strands of DNA molecule
(d) both (a) and (c).

(ii) Which of the following ions are used by restriction endonucleases for restriction?
(a) Mg^{2+} ions (b) Mn^{2+} ions (c) Na^{+} ions (d) K^{+} ions

(iii) Restriction endonuclease was isolated for the first time in a
(a) plant cell (b) animal cell (c) prokaryotic cell (d) germinal cell.

(iv) Restriction endonucleases are also called as molecular or biological scissors because
(a) they cleave base pairs of DNA only at their terminal ends
(b) they cleave one or both the strands of DNA
(c) they act only on single stranded DNA
(d) none of these.

(v) Select the option that correctly states the working action of restriction endonuclease *Eco*R 1 on DNA sequence GAATTC.

(a)
$$\begin{array}{c} \downarrow \\ 5'-GAATTC-3' \\ 3'-CTTAAG-5' \\ \uparrow \end{array}$$

(b)
$$\begin{array}{c} \downarrow \\ 5'-GAATTC-3' \\ 3'-CTTAAG-5' \\ \uparrow \end{array}$$

(c)
$$\begin{array}{c} \downarrow \\ 5'-GAATTC-3' \\ 3'-CTTAAG-5' \\ \uparrow \end{array}$$

(d)
$$\begin{array}{c} \downarrow \\ 5'-GAATTC-3' \\ 3'-CTTAAG-5' \\ \uparrow \end{array}$$

3

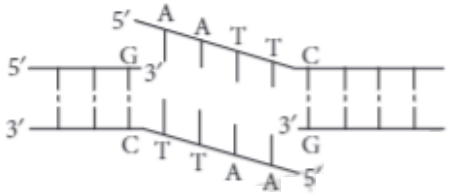
Read the following and answer any four questions from 3(i) to 3(v) given below:
Tools used in the formation of recombinant DNA are of three types. These are enzymes, cloning vectors and competent host. Lysing enzymes are used to extract DNA for experimental purpose from the cells. Cleaving enzymes break the DNA molecules. They are of three types : exonucleases, endonucleases and restriction endonucleases. A competent host is required for transformation with recombinant DNA and cloning vectors help to propagate DNA.

(i) Which of the following is an example of natural lysing activity in a human body?
(a) Lysozyme present in tears dissolve the bacterial cell wall.
(b) Conversion of starch to maltose in the buccal cavity
(c) Absorption of digested food into the intestinal cells.
(d) Conversion of protein molecules into amino acids in the stomach.

(ii) Which of the following depicts exonuclease activity?

(a) 

(b) 

(c) 

(d) All of these

(iii) Cloning vectors are the DNA molecules that

(a) carry foreign DNA segment but do not replicate inside the host cell
(b) carry foreign DNA segment and replicate inside the host cell
(c) transfer nuclear DNA form nucleus to the cytoplasm of the same cells
(d) help in sealing gaps in DNA segments.

(iv) Transfer of DNA into a eucaryotic cell is called

(a) transformation (b) transduction (c) transfection (d) electroporation.

(v) **Assertion :** Type I restriction enzymes are not used in rDNA technology.
Reason : Type I restriction endonucleases consist of two different subunits and require ATP for restriction activity.

(a) Both assertion and reason are true and reason is the correct explanation of assertion.
(b) Both assertion and reason are true but reason is not the correct explanation of assertion.
(c) Assertion is true but reason is false.
(d) Both assertion and reason are false.

4

Read the following and answer any four questions from 4(i) to 4(v) given below:

The foundations of recombinant DNA (rDNA) were laid by the discovery of restriction enzymes. These enzymes are present in many bacterias where they function as a part of their defense mechanism called the Restriction Modification system (RM system). Molecular basis of this system was explained first by Werner Arber in 1962. The Restriction Modification system consists of two components:

- A restriction enzyme (called restriction endonuclease) identifies the introduced foreign DNA and cuts it into pieces.
- The second component is a modification enzyme (methylase) that adds a methyl group to DNA at specific site to protect it from the restriction enzyme cleavage.

(i) Restriction endonucleases are enzymes present in __(i)__ where they function as a part of __(ii)__ mechanism.

(a) (i) bacteria (ii) digestive (b) (i) protists (ii) transcription
(c) (i) plant cells (ii) replication (d) (i) prokaryotes (ii) defence

(ii) Which of the following statements regarding modification enzyme is correct?

(a) It adds methyl group to one or two bases usually within the host DNA sequence to protect it from the restriction enzyme.
(b) It adds ethyl group to one or two bases usually within the sequence recognised by the restriction enzymes.
(c) It adds methyl group to only one of bases within the foreign DNA sequence that is recognised by the restriction enzymes.
(d) None of these

(iii) Which of the following is a type II restriction enzyme?
(a) *Alu* I (b) *EcoR* I (c) *BamH* I (d) All of these

(iv) Which of the following is the first discovered restriction endonuclease?
(a) *Sal* I (b) *EcoR* I (c) *Hind* II (d) *EcoR* II

(v) Components of Restriction Modification System include
(a) restriction enzyme (b) modification enzyme (c) lysing enzyme (d) both (a) and (b).

5

Read the following and answer any four questions from 5(i) to 5(v) given below:

In recombinant DNA technology, the fragments of DNA generated after cutting the DNA by restriction enzymes are separated according to their size or length by gel electrophoresis. Gel electrophoresis is performed in a gel matrix so that molecules of similar electric charges can be separated on the basis of size. Most commonly used matrix in gel electrophoresis is agarose. The fragments are separated under the influence of electric field. The separated DNA fragments can be seen only after staining the DNA with compound known as ethidium bromide (EtBr) followed by exposure to UV radiation as bright orange band.

(i) Gel electrophoresis is used for the separation of
(a) DNA only (b) DNA and RNA only
(c) DNA and proteins only (d) DNA, RNA and proteins.

(ii) Most commonly used matrix is __(i)__ which is a __(ii)__ extracted from __(iii)__ .
(a) (i) agarose (ii) polysaccharide (iii) sea weed (b) (i) agarose (ii) protein (iii) sea weed
(c) (i) EtBr (ii) polysaccharide (iii) sea weed (d) (i) EtBr (ii) protein (iii) bacteria

(iii) A DNA molecule was treated with a restriction endonuclease and three fragments of size (i) 426 kb, (ii)129 kb and (iii) 46 kb were obtained. Identify the order in which these bands will arrange themselves in the gel plate after gel electrophoresis is completed. (Assuming that negative part of electrode is towards the well)
(a) (iii) → (ii) → (i) (b) (i) → (ii) → (iii) (c) (i) → (iii) → (ii) (d) (iii) → (i) → (ii)

(iv) Which of the following statements regarding gel electrophoresis is incorrect?
(a) Separated DNA fragments can be seen only after staining DNA with EtBr.
(b) DNA fragments are separated according to their size.
(c) Under the influence of electric field, positively charged molecules move towards the anode and negatively charged molecules move towards the cathode.
(d) None of these

(v) The factor that will not affect the rate of DNA migration in gel electrophoresis is
(a) size of DNA molecule (b) concentration of DNA
(c) voltage supplied (d) concentration of the gel.

6

Read the following and answer any four questions from 6(i) to 6(v) given below:

Rama lives in a society where a robbery occurred last night. Robbers came into the flat and murdered the old lady residing there. Police came and restricted the entry into the flat. They took samples from the room, where the dead body was found. While examining, they found that there is some blood and tissue in the nails of old

lady. According to their observation, police filtered out their inspection to three suspects *viz.* servant, cook and milkman. Finally after two days of robbery, police caught the criminal. It was the old lady's cook. Rama was amazed to see that how quickly police completed and shut the case. She asked the inspector that how they did it? The police man told her that it become possible due to the sample collected from the victim, that lead them to the criminal. The sample taken from nail scraping was amplified using PCR and then tested.

(i) What technique was used by the police to identify the criminal?

(a) DNA fingerprinting (b) Gel electrophoresis

(c) Molecular diagnosis (d) Clonning

(ii) In PCR, the temperature used to denature the DNA is about

(a) 76° C (b) 25°C (c) 95°C (d) 40°C.

(iii) Which of the following statements regarding PCR is correct?

(a) Taq polymerase, which is isolated from bacterium *Thermus aquaticus* is stable at low temperature only.

(b) With the help of DNA ligase, the complementary sticky ends of the DNA are joined to produce a *r*DNA.

(c) Since the sequence of primers are complementary to 5′ end of the template DNA, they anneal to it.

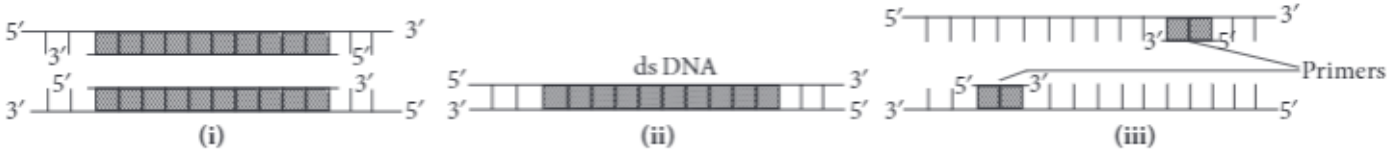
(d) DNA purified from the cell is precipitated by adding hot ethanol.

(iv) Taq polymerase synthesises DNA region between the primers using

(a) Mg^{2+} (b) dNTPs

(c) DNA ligase (d) both (a) and (b).

(v) Given below are steps of polymerase chain reaction.



Select the option that correctly mention the sequence in which they occur.

(a) (ii) → (iii) → (i) (b) (i) → (ii) → (iii)

(c) (iii) → (i) → (ii) (d) (ii) → (i) → (iii)

7

Read the following and answer any four questions from 7(i) to 7(v) given below:

The vectors are DNA molecules that can carry a foreign DNA segment and replicate inside the host cell. Vectors may be plasmids, bacteriophages (viruses that attack bacteria), cosmids, yeast artificial chromosomes (YACs), Bacterial artificial chromosomes (BACs) and viruses. The most widely used, versatile, easily manipulated vector pBR 322 is an ideal plasmid vector. Features that are required to facilitate cloning into a vector includes origin of replication (*Ori*) which is a specific sequence of DNA bases responsible for initiating replication, selectable marker genes and cloning sites.

(i) p in pBR 322 denotes that it is a

(a) plasmid (b) prokaryote

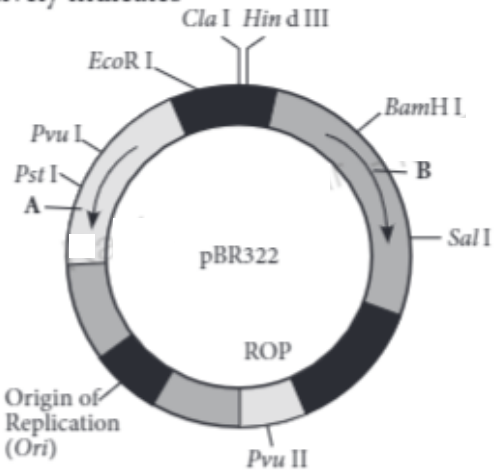
(c) protist (d) plant cell.

(ii) *Ori* is a specific DNA sequence that help in

(a) attachment of primers (b) initiation of replication

(c) extension of DNA base (d) initiation of denaturation.

(iii) A and B shown in the figure respectively indicates



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

(iv) Selectable markers in vector
(a) are responsible for replication
(b) help in selecting transformants from non-transformants
(c) code for proteins involved in the replicating plasmids
(d) contain unique recognition sites.

(v) Plasmid vectors are
(a) dsDNA molecule
(b) extra-chromosomal
(c) present in bacteria and yeast
(d) all of these.

8

Read the following and answer any four questions from 8(i) to 8(v) given below:

Rajat is a student of biotechnology. His professor tells him that for transformation with recombinant DNA the bacterial cells must be made capable of taking up DNA as DNA do not pass through membrane. While doing experiment in the lab, Rajat noticed that bacterial cells were not taking up the foreign DNA even after treating it with sodium ion. He asked his professor, the reason behind this. His professor explained that he should check the valency and charge of the ion that he is using for the treatment.

(i) It is difficult for DNA to pass through the membrane as
(a) it is a hydrophilic molecule
(b) it is a hydrophobic molecule
(c) it is a circular molecule
(d) it changes its shape when it comes in contact with host cell.

(ii) What type of ions are used for DNA mediated gene transfers?
(a) Divalent anions
(b) Divalent cations
(c) Monovalent cations
(d) Monovalent anions

(iii) *r*DNA stands for
(a) reduced DNA
(b) red DNA
(c) recombinant DNA
(d) related DNA.

(iv) Which of the following statements with regard to DNA is correct?

(a) DNA is a positively charged molecule having two polynucleotide chains.

(b) Nitrogen bases of two polynucleotide chain form complementary pairs, *i.e.*, A opposite G and T opposite C.

(c) Backbone of DNA chain is built up of alternate deoxyribose sugar and phosphate group.

(d) Both (a) and (c)

(v) **Assertion :** Competent host is essential for transformation with *r*DNA.

Reason : Transfer of DNA in a prokaryotic cell is called transfection.

(a) Both assertion and reason are true and reason is the correct explanation of assertion.

(b) Both assertion and reason are true but reason is not the correct explanation of assertion.

(c) Assertion is true but reason is false.

(d) Both assertion and reason are false.

9

Read the following and answer any four questions from 9(i) to 9(v) given below:

Bioreactors are considered as vessels in which raw materials are biologically converted into specific products by microbes, plant and animal cells or their enzymes. They are used for large scale production as they provide optimum growth conditions such as temperature, *p*H, substrate, vitamins, oxygen and salts for obtaining desired product. Most commonly used bioreactors are of stirring type which include simple stirred tank bioreactor and sparged stirred-tank bioreactor.

(i) Bioreactor are useful in

(a) amplifying a gene

(b) isolation of genetic material

(c) processing large volume of culture

(d) infecting DNA in a cell.

(ii) Which of the following is essential to obtain desired product in a bioreactor?

(a) Size of the bioreactor

(b) Sterile condition

(c) Quantity of the raw material

(d) All of these

(iii) **Assertion :** The stirred-tank is well suited for large scale production of microorganisms under aseptic conditions.

Reason : In sparged stirred tank bioreactor, surface area for oxygen transfer is increased.

(a) Both assertion and reason are true and reason is the correct explanation of assertion.

(b) Both assertion and reason are true but reason is not the correct explanation of assertion.

(c) Assertion is true but reason is false.

(d) Both assertion and reason are false.

(iv) Growth condition that could affect the quality of obtained product in a bioreactor are

(a) temperature and pH only

(b) pH and oxygen supply only

(c) temperature and oxygen supply only

(d) temperature, pH and oxygen supply.

(v) Vessels in which raw materials are biologically converted into specific products are

(a) bioreactors

(b) fermentors

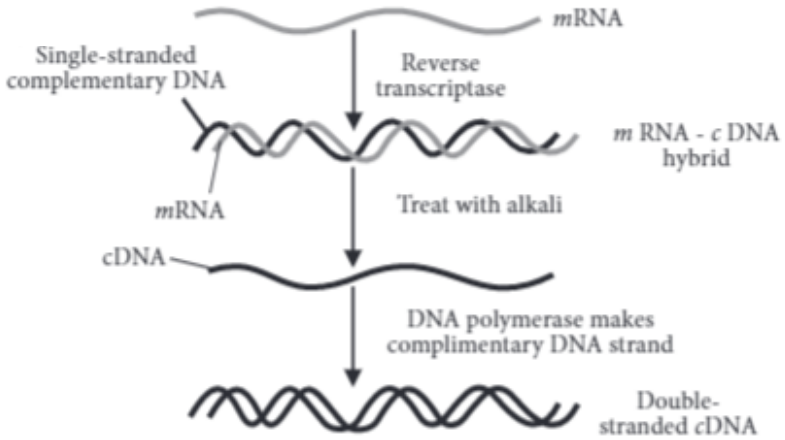
(c) gene guns

(d) both (a) and (b).

10

Read the following and answer any four questions from 10(i) to 10(v) given below:

The DNA, which is transferred from one organism into another by joining it with the vehicle DNA is called passenger or foreign DNA. Generally three types of passenger DNAs are used. These are complementary DNA (*c*DNA), synthetic DNA (*s*DNA) and random DNA. Complementary DNA (*c*DNA) is synthesized on RNA template (usually *m*RNA) with the help of reverse transcriptase. Synthetic DNA (*s*DNA) is synthesized on DNA template or without a template. Random DNA are small fragments formed by breaking a chromosome of an organism in the presence of restriction endonucleases.



(i) Reverse transcriptase enzyme was discovered by

(a) Temin and Baltimore (b) Cohen and Boyer

(c) Arber and Nathan (d) Paul Berg.

(ii) During *c*DNA formation, what would happen if DNA formed by reverse transcriptase is not treated with the alkali?

(a) *c*DNA will not be digested

(b) *m*RNA will not be digested

(c) Hydrogen bonds will not form between base pairs

(d) *m*RNA will not be formed.

(iii) Enzyme that helps in the formation of double stranded *c*DNA is

(a) DNA synthetase (b) ligase (c) DNA polymerase (d) helicase.

(iv) DNA polymerase can be obtained form

(a) retrovirus (b) *Agrobacterium*

(c) tobacco mosaic virus (d) *Thermus aquaticus.*

(v) DNA synthesised without a template is referred to as

(a) complementary DNA (b) random DNA (c) synthetic DNA (d) Z-DNA.

ASSERTION & REASON

For question numbers 11-25, two statements are given-one labelled Assertion and the other labelled Reason. Select the correct answer to these questions from the codes (a), (b), (c) and (d) as given below.

(a) Both assertion and reason are true and reason is the correct explanation of assertion.

(b) Both assertion and reason are true but reason is not the correct explanation of assertion.

(c) Assertion is true but reason is false.

(d) Both assertion and reason are false.

11. **Assertion :** Bacterial cells are made competent by treating them with specific concentration of a divalent cation.
Reason : Treatment of bacterial cell with a divalent cation increases the efficiency with which DNA enters the bacterium through pores in its cell wall.

12. **Assertion :** Both the passenger and vehicle DNAs are treated separately with separate restriction endonuclease.
Reason : Ligation is done by the use of alkaline phosphatase and DNA ligase.

13. **Assertion :** Vector DNA and foreign DNA are cut by same restriction endonuclease.
Reason : Digestion of vector DNA and foreign DNA with same enzyme produces complementary sticky ends.

14. **Assertion :** Selectable marker is meant for distinguishing a recombinant from non-recombinant.
Reason : Every recombinant can flourish in medium having both ampicillin and tetracycline, while the non- recombinants cannot.

15. **Assertion :** Restriction endonuclease recognises palindromic sequence in DNA and cuts them.
Reason : Palindromic sequence has two unique recognition sites *Pst* I and *Pvu* I recognised by restriction endonuclease.

16. **Assertion :** Bacteriophage vectors are more advantageous than plasmid vectors.
Reason : Bacteriophage vectors can be easily detected at the time of cloning experiments.

17. **Assertion :** Type I restriction endonucleases are not used in recombinant DNA technology.
Reason : Type I restriction endonucleases recognise specific sites within the DNA but do not cut these sites.

18. **Assertion :** YAC vectors have been exploited extensively in the mapping of large genomes.
Reason : YAC vectors have a composite structure made of bacteriophage and plasmid.

19. **Assertion :** cDNA is copy DNA which is synthesised *in vivo* on a DNA template using DNA polymerase.
Reason : cDNA of all possible genes are ligated with different plasmids and maintained in either plant or animal cells.

20. **Assertion :** Amplification of a gene of interest can be done by polymerase chain reaction.
Reason : It is possible to amplify DNA segment approximately 1 billion times within a span of one day.

21. **Assertion :** In recombinant DNA technology, human genes are often transferred into bacteria (prokaryotes) or yeast (eukaryote).
Reason : Both bacteria and yeast multiply very fast to form huge populations which express the desired gene.

22. **Assertion :** Restriction enzymes cut the strand of DNA to produce sticky ends or blunt ends.
Reason : Stickiness of the ends facilitates the action of the enzyme DNA polymerase.

23. **Assertion :** DNA fingerprinting involves identifying differences in specific regions of DNA sequence.
Reason : DNA fingerprinting is the basis of paternity testing.

24. **Assertion :** Soil inhabiting bacterium *Agrobacterium tumefaciens* is called a natural plant genetic engineer.
Reason : *Agrobacterium tumefaciens* produce crown galls in several dicot plants.

25. **Assertion :** The insertion of DNA fragment into pBR 322 plasmid using enzyme *Pst* I or *Pvu* I make ampicillin resistant gene non functional.
Reason : Bacterial cells containing recombinant pBR322 is unable to grow in the presence of ampicillin.

HINTS & EXPLANATIONS

1. **(i) (d)**
(ii) (b) : Plasmid in a bacterial cell is an extra chromosomal material that undergo replication with or without chromosomal DNA.
(iii) (a)
(iv) (c) : In 1972, Paul Berg was able to introduce a gene of SV-40 virus into a bacterium with the help of lambda phage.
(v) (b)

2. **(i) (d) :** Only type II restriction enzymes are used in gene manipulation for two reasons : (a) No ATP is required for the cleaving action. (b) It makes cleavage or cut in both the strands of DNA molecule.
(ii) (a) : All the three types of restriction endonucleases require Mg^{2+} ions for restriction.
(iii) (c) : Restriction endonuclease was isolated for the first time by W. Arber in 1962 in bacteria.
(iv) (b)
(v) (b)

3. **(i) (a) :** Conversion of starch into maltose and protein into amino acids is due to hydrolysis.
(ii) (a) : Exonuclease removes nucleotides from the terminal ends (either 5′ or 3′) of DNA in one strand of duplex.
(iii) (b)
(iv) (c)
(v) (c) : Type I restriction endonuclease consist of three different subunits and requires ATP, Mg^{2+}, S-adenosyl methionine for restriction.

4. **(i) (d)**
(ii) (a)
(iii) (d) : Different examples of Type II restriction endonuclease are *Alu* I, *EcoR* I, *BamH* I, etc.
(iv) (c)
(v) (d) : The restriction modification system consists of two components (i) A restriction enzyme called restriction endonucleases which identifies the introduced foreign DNA and cuts it into pieces and (ii) A modification enzyme (methylase) that adds a methyl group to DNA at a specific site to protect the site from restriction endonuclease cleavage.

5. **(i) (d) :** Gel electrophoresis is a technique used to separate fragments of molecules, *i.e.*, DNA, RNA and protein.
(ii) (a)
(iii) (b)
(iv) (c) : Under the influence of electric field positively charged molecules move towards the cathode and negatively charged molecules move towards the anode.
(v) (b) : Concentration of DNA will not affect the migration of DNA molecule in a gel electrophoresis. As in gel electrophoresis, molecules separate according to their size therefore DNA size will affect migration. Increased voltage supply will increase rate of migration and the more concentrated gel will reduce rate of migration.

6. **(i) (a) :** DNA fingerprinting is one of highly accurate application of biotechnology. It is helpful in solving crime, legal disputes, establishing identity of criminal or parents, etc.
(ii) (c) : In PCR, during denaturation, the target DNA is heated at high temperature resulting in the separation of the two strands.
(iii) (b) : Taq polymerase isolated from bacterium *Thermus aquaticus* is stable at high temperature. Sequence of primers are complementary to 3′ end of the template. Purified DNA is precipitated by adding chilled ethanol.
(iv) (d)
(v) (a) : Three steps of PCR are : denaturation, annealing and extension.

7. **(i) (a) :** In *pBR*322 plasmid, P – denotes that it is a plasmid; BR – stands for Boliver and Rodriguez, who constructed this plasmid; 322 – is a number given to distinguish this plasmid from others developed in the same laboratory.
(ii) (b)
(iii) (c)
(iv) (b) : Plasmid pBR322 has two resistance gene, *i.e.*, ampicillin resistance (amp^R) and tetracyclin resistance (tet^R) which are considered useful for selectable markers.
(v) (d)

8. **(i) (a) :** DNA is a hydrophilic molecule therefore it cannot pass through membrane.
(ii) (b) : Divalent cations, such as calcium increases the efficiency with which DNA enters the bacteria through pores in its walls.
(iii) (c)
(iv) (c) : DNA is a negatively charged molecule and in a DNA molecule nitrogen bases form complementary pairs, *i.e.*, A opposite T and C opposite G.
(v) (c) : Transfer of DNA in a prokaryotic cell is called transformation.

9. **(i) (c) :** Bioreactors are considered as vessels in which raw molecules are biologically converted into specific products.
(ii) (b) : In a bioreactor, all operations must be carried under sterile conditions to avoid contamination.
(iii) (b)
(iv) (d) : A bioreactor provides the optimal growth conditions such as temperature, *p*H, substrate, vitamins, oxygen and salts for obtaining the desired product.
(v) (d)

10. **(i) (a)**
(ii) (b) : The *c*DNA formation involves the alkaline denaturation of the *m*RNA-*c*DNA hybrid. The double stranded DNA molecule formed after the activity of reverse transcriptase is treated with alkali to digest *m*RNA.
(iii) (c) : A *c*DNA strand is formed on the separated single stranded DNA template with the help of DNA polymerase enzyme.

(iv) (d)
(v) (c)

11. (a): The bacterial cell is made competent by treating it with specific concentration of a divalent cation such as calcium to increase the efficiency with which DNA can enter the bacteria through pores of cell wall because DNA is a hydrophilic molecule and it cannot pass through cell membrane so making bacterial cell competent ease the process to take up DNA.

12. (d)

13. (a)

14. (c): Selectable markers are the substances which helps in identifying recombinants and eliminating non recombinants. Generally the genes encoding the resistance to antibiotics such as tetracycline, ampicillin, kanamycin or chloramphenicol, etc. are useful selectable markers for *E.coli*.

15. (c): A palindromic sequence in DNA is one in which the 5′ to 3′ base pair sequence is identical on both strands. Restriction enzymes recognise and make a cut within specific palindromic sequence, known as restriction sites, in the DNA. This is usually a 4 or 6 base pair sequence.

16. (a): Bacteriophage vector have two advantages over plasmid vectors.
(i) They are more efficient than plasmids for the cloning of large DNA fragments. For example, the largest cloned insert in lambda phage is 24kb while for plasmid vector it is less than 15 kb.
(ii) It is easier to screen a large number of phage plaques than bacterial colonies for identification of recombinant vectors.

17. (a): Restriction endonuclease are enzymes that cleaves DNA at specific sites along the molecule. Type I restriction enzyme recognises specific DNA sequences but make their cut at seemingly random sites that can be as far as 1000 base pair away from the recognition site.

18. (c): Yeast Artificial Chromosome (YACs) is a human engineered DNA molecule derived from the yeast. YACs are often used in connection with mapping and sequencing of genomes. Segments of an organism's DNA, upto one million base pairs in length, can be inserted into YACs. The YACs are then taken up by yeast cells. As the yeast cell grow and divide, they amplify the YAC DNA, which can be isolated and used for DNA mapping and sequencing.

19. (d)

20. (b)

21. (a): In recombinant DNA technology, widely used host for replication and amplification of recombinant DNA are prokaryotic *E. coli* and the eukaryotic yeast. They replicate very fast to form a large population which expressed desired gene. Yeast artificial chromosome (YAC) are important cloning tools for the analysis of complex genome such as that of humans. They allow the maintenance, propagation and analysis of such genome in an experimentally tractable system, the yeast.

22. (c): Restriction enzyme, a type of endonuclease, functions by "inspecting" the length of a DNA sequence. Once it finds a recognition sequence, it binds and cut each of the two strands of the double helix at specific point a staggered cut generates two sticky ends and a straight cut generates blunt end. The staggered cut leaving single stranded portions at the ends which results in overhanging stretches called sticky ends. These are named so because they form hydrogen bonds with their complementary counter parts, *i.e.*, they can join similar complementary ends of DNA fragment from some other source with the help of DNA ligase. This stickiness of the ends facilitates the action of the enzyme DNA ligase, not DNA polymerase.

23. (b) : DNA fingerprinting involves identifying differences in some specific regions in DNA sequence called as repetitive DNA, because in these sequences, a small stretch of DNA is repeated many times. These sequences normally do not code for any proteins, but they form a large portion of human genome. These sequence show high degree of polymorphism and form the basis of DNA fingerprinting. As the polymorphisms are inheritable from parents to children, DNA fingerprinting is the basis of paternity testing in case of disputes.

24. (b)

25. (b): Plasmid pBR322 has a variety of unique restriction sites for restriction endonucleases. Two unique sites, *Pst I* and *Pvu I* are located within the amp^r gene and *BamHI*, *Sal I* etc. are within tet^r gene. The presence of restriction sites within the marker tet^r and amp^r permits an easy selection for cell transformed with the recombinant pBR322. Insertion of the DNA fragment into the plasmid using enzyme *Pst* I and *Pvu* I places the DNA insert within the gene amp^r and make it non functional.