CHAPTER › 11

Biotechnology : Principles and Processes

- **Biotechnology** is the technique of using living organisms or enzymes from organisms to produce products and processes useful to humans.
- Biotechnology deals with the large scale production and marketing of products such as enzymes, insulin or antibiotics, etc., that are of importance to mankind.
- **The European Federation of Biotechnology** (EFB) has given a definition of biotechnology as, the integration of natural science and organisms cells, parts thereof and molecular analogues for products and services'.

Principles of Biotechnology

Following two core techniques gave birth to modern biotechnology

- **Genetic engineering** It is the alteration of the chemistry of genetic material (DNA/RNA), introduce these into host organisms and consequently change the phenotype of the host organism.
- **Bioprocess engineering** It is the maintenance of sterile conditions in order to enable the growth of only desired microbes or eukaryotic cells in large quantities for the production of antibiotics, enzymes, hormones, vaccines, etc.

Genetic Engineering

- It is the deliberate modification of an organism's DNA, using various techniques. This altered DNA (recombinant DNA) is then introduced into host organisms to change their phenotype.
- This is followed by growing this genetically modified cell in large quantities, by maintaining sterile environment, for the manufacture of biotechnological products like antibiotics, vaccines, enzymes, etc.
- Techniques of genetic engineering include construction of **recombinant DNA**, **gene cloning** and **gene transfer**. These techniques allow the isolation and introduction of a set of desirable genes without introducing undesirable genes into the target organism.
- **Origin of replication** (*ori*) is a specific DNA sequence in the chromosome which can initiate DNA replication. The foreign DNA introduced into the host genome has to be linked the origin of replication in the host chromosome for the gene to be able to multiply. This is also known as **cloning** which involves making multiple identical copies of any template DNA. If the foreign gene is not linked to the *ori* sequence it may not be able to multiply.
- In 1972, the first recombinant DNA was constructed by **Stanley Cohen** and **Herbert Boyer**. They isolated the antibiotic resistance gene by cutting out a piece of DNA from a **plasmid** (autonomously replicating circular extra-chromosomal DNA) of *Salmonella typhimurium*. The cutting of DNA at specific locations become possible with the help of **restriction enzymes** (molecular scissors).
- The cut pieces of DNA were then linked with the plasmid DNA using DNA ligase enzyme. These plasmids act as **vectors** to transfer the piece of DNA attached to it into the host organism. This makes a new circular autonomously replicating DNA created *in vitro* and is known as the **recombinant DNA**.
- The recombinant DNA is transferred into *Escherichia coli*, a bacterium closely related to *Salmonella*, where it replicates using the new host's DNA polymerase enzyme and makes multiple copies of itself. It also produces multiple copies of the antibiotic resistance gene in the new host (*E. coli*). This process is called as **cloning** of antibiotic resistance gene in *E. coli*.

- There are three basic steps involved in genetically modifying an organism
  - Identification of DNA with desirable genes.
  - Introduction of the identified DNA into the host.
  - Maintenance of introduced DNA in the host and transfer of the DNA to its progeny.

Tools of Recombinant DNA Technology

Genetic engineering or recombinant DNA technology can be accomplished only if we have the key tools, i.e. restriction enzymes, polymerase enzymes, ligases, vectors and the host organism.

1. Restriction Enzymes

- In the year 1963, the two enzymes responsible for restricting the growth to bacteriophage in *E. coli* were isolated. One of these added methyl group to DNA, while the other cut DNA. The latter was called **restriction endonuclease**.
- The first restriction endonuclease, i.e. *Hin*d II was isolated by **Smith Wilcox** and **Kelley** in 1968.
  *Hin*d II always cut the DNA at specific base sequences, i.e. of six base pairs. Apart from *Hin*d II, more than 900 restriction enzymes have been isolated from over 230 strains of bacteria.
- Naming of restriction enzyme proceeds in a way that the first letter of the name comes from the genus and the second two letters come from the species of prokaryotic cell. Roman number following the names indicate the order in which the enzyme were isolated from that strain of bacteria, e.g. *Eco* RI comes from *E. coli* RY13.
- Restriction enzymes belong to a larger class of enzymes called **nucleases**, which are of the following two types
  - **Exonucleases**, which remove nucleotides from the ends of the DNA (either 5′ or 3′) in one strand of duplex.
  - **Endonucleases** make cuts at specific positions within the DNA by inspecting the length of a DNA sequence. Once finds its specific **recognition sequence**, it will bind to the DNA and cut each of the two strands of the double helix at specific points in their sugar phosphate to backbones.
- These restriction enzymes recognise a **palindromic nucleotide sequence** in the DNA and cut both the strands of DNA at that point. Palindrome in the 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 sequence reads the same on the two strands whether read in 5′ → 3′ direction or 3′ → 5′ direction.

5′–GAATTC–3′
3′–CTTAAG–5′

Types of Restriction Enzyme

- The restriction enzymes can be of three types, on the basis of their chemical and physiological properties.

Features	Type I Enzyme	Type II Enzyme	Type III Enzyme
Protein structure	Bifunctional enzyme with 3 subunits	Separate endonuclease and methylase	Bifunctional enzyme with 2 subunits
Recognition site	Bipartite and asymmetrical (e.g. TGAC and TGCT)	Short sequence (4-6 bp), often palindromic	Asymmetrical sequence of 5-7 bp
Cleavage site	Non-specific >1000 bp from recognition site	Same as or close to recognition site	24-26 bp downstream of recognition site
Restriction and methylation	Mutually exclusive	Separate reactions	Simultaneous
ATP needed for restriction	Yes	No	Yes
Mg^{2+}needed for restriction	Yes	Yes	Yes
Commonly used in	Random cutting and fragments making	Gene cloning	Gene cloning

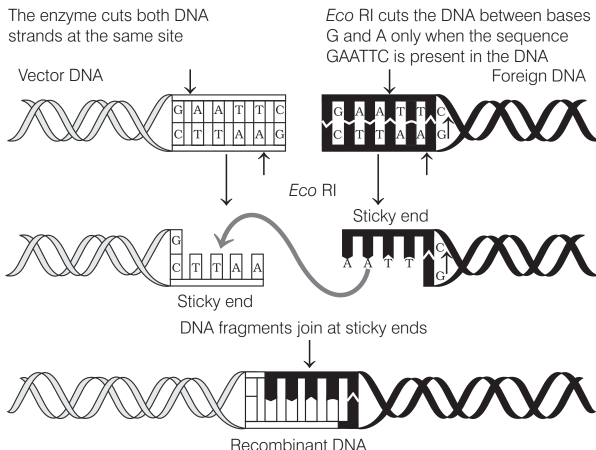
Examples of Restriction Enzymes

Names	Sources	Sites	Types of End
Hpa I	*Haemophilus parainfluenzae*	5′ GTT - AAC 3′ 3′ CAA - TTG 5′	Blunt
Ssp I	*Sphaerotilus* species	5′ AAT - ATT 3′ 3′ TTA - TAA 5′	Blunt
Pst I	*Providencia stuartii*	5′ CTGCA - G 3′ 3′ G - ACGTC 5′	Sticky
Hind II	*Haemophilus influenzae*	5′ GTC - GAC 3′ 3′ CAG - CTG 5′	Blunt
Eco RI	*Escherichia coli*	5′ G - AATTC 3′ 3′ CTTAA - G 5′	Sticky
Hae III	*Haemophilus aegyptius*	5′ GG - CC 3′ 3′ CC - GG 5′	Blunt
Bam HI	*Bacillus amyloliquefaciens*	5′ GGAT - CC3′ 3′ CCTA - GG 5′	Sticky

- Restriction enzymes cut the strand of DNA, a little away from the center of the palindrome sites, but between the same two bases on the opposite strands. These staggered cuts leave single-stranded portions at both the ends. These are referred to as **sticky ends**.
- There are other restriction enzymes which cut both the DNA strands at the same place so that single-stranded pieces are not left in the ends. Such ends are called **blunt ends**.
- Stickiness is the chemical ability of a DNA molecule to base pair with any other DNA molecule that has also been cut by the same restriction enzyme. It means it will have

same sequence hanging unpaired. This stickiness of the ends facilitates the action of the enzyme **DNA ligase**.

- **DNA ligase** (molecular binder) enzymes help in sealing the gaps in DNA fragments by forming a phosphodiester bond between the adjacent 3′ — OH and 5′ phosphate terminals, thereby joining nicks in double-stranded DNA.
- **Lysing enzymes/Lyases** enzymes are used for the isolation of DNA from cells, e.g. lysozyme is used to digest the bacterial cell wall for the extraction of cellular DNA. Protease, lipase and other degrading enzymes come in this category.
- **Alkaline phosphatase** catalyse the removal of 5′ phosphate group from the DNA and thus, modify the terminus of DNA.



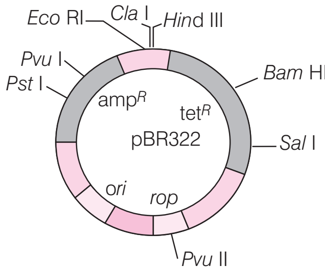
Steps in the formation of recombinant DNA by action of restriction endonuclease enzyme *Eco* RI

Separation and Isolation of DNA Fragments

- Separation of restriction fragments from each other can be done by **gel electrophoresis**.
- An electric field is applied and these fragments are forced to move through a viscous gel of agarose (a natural polymer extracted from sea weeds).
- Since, DNA fragments are negatively charged (because of their phosphate groups) they will move towards the positively charged pole, i.e. anode.
- Smaller fragments move faster and bigger ones move slowes, but they all separate out (according to their size) into bands that can be identified later by staining.
- The staining of DNA bands can be done with a compound known as ethidium bromide followed by exposure to UV radiation. Bright orange coloured bands of DNA are visible.
- The DNA fragments separated and cut out from the gel and extracted from gel piece. This step is known as **elution**.

2. Cloning Vectors

- Plasmids and bacteriophages are used as **cloning vector**s. This is because plasmids and bacteriophages have the ability to replicate within the bacterial cell independent of chromosomal DNA.
- Bacteriophages have very high copy numbers of their genome within the bacterial cells. But in case of plasmids, some may have only one or two copies per cell whereas others may have 15-100 copies per cell.
- Cloning vectors use the machinery of bacterial cell to replicate and thereby, increase the copy number (make clones) of the DNA inserted into them.
- These help easy linking of foreign DNA and allow the selection of recombinants (bacterial cells that have picked up recombinant plasmid) from non-recombinants (those who have not).
- All vectros have four special features that are required to facilitate cloning into a vector.
- **Origin of replication** (*ori*) is the 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 also controls the copy number of the linked DNA.
- **Selectable marker** helps in identifying and eliminating non-transformants and selectively permitting the growth of the transformants.
  - **Transformation** is the procedure through which a piece of DNA is introduced into a host bacterium.
  - Normally, antibiotics such as ampicillin, chloramphenicol, tetracycline or kanamycin, etc., are considered useful selectable markers.
- **Cloning sites** are used to link the alien DNA. The vector needs to have cloning or **recognition sites** for the commonly used restriction enzymes. The presence of more than one recognition sites within the vector will generate several fragments and complicate the gene cloning.



E. coli cloning vector pBR322 showing restriction sites (*Hin*d III, *Eco*RI, *Bam*HI, *Sal* I, *Pvu* II, *Pst* I, *Cla* I), *ori* and antibiotic resistance genes (amp^R and tet^R). *rop* codes for the proteins involved in the replication of the plasmid

- The ligation of foreign DNA is carried out at a restriction site present in one of the two **antibiotic resistance** genes. It can be done at the *Bam* HI site of tetracycline resistance gene in the vector pBR 322.
- In this case, the **recombinant plasmids** will **lose tetracycline resistance** due to the insertion of foreign DNA but can still be selected out from non-recombinant ones by plating the transformants on a medium containing ampicillin.
- The recombinants will grow in ampicillin containing medium but not on that containing tetracycline. But, non-recombinants will grow on the medium containing both the antibiotics.
- Other selectable markers can differentiate recombinants from non-recombinants on the basis of their ability to produce colour in the presence of a chromogenic substrate. In this, recombinant DNA is inserted within the coding sequence of an enzyme, β-galactosidase. This results into inactivation of the gene synthesis of this enzyme and referred to as **insertional inactivation**.
- The presence of a chromogenic substrate gives blue coloured colonies if the plasmid in the bacteria does not have an insert and if the colonies have inserted plasmid, such colonies are recombinants and show no colouration.

- **Vectors for cloning genes in plants and animals** A pathogen of several dicot plant is able to deliver a piece of DNA, i.e. 'T-DNA' to transform normal plant cells into a tumour cells and disect these tumour cells to produce the chemicals required by the pathogen. Similarly, retrovirus in animals have the ability to transform normal cells into **cancerous cells**.
- The tumour inducing (Ti) plasmid of *Agrobacterium tumefaciens* has now been modified into a cloning vector which is able to use the mechanisms to deliver genes of our interest into a variety of plants.
- Similarly, retroviruses can be disarmed and used to deliver desirable genes into animal cells.

3. Competent Host

- Since, DNA is a hydrophilic molecule, it cannot pass through cell membrane that has hydrophobic ends both on the inside and outside. Hence, various artificial means have to be used to make the cells competent to take up foreign DNA.
- Bacteria are treated with a specific concentration of a divalent cation, such as calcium, which makes the cell membrane more permeable. Recombinant DNA can then be forced into such cells by incubating the cells with recombinant DNA on ice, followed by placing them briefly at 42°C (heat shock) and then putting them back on ice. This makes the bacterial cell competent to take up the recombinant DNA.
- Recombinant DNA is directly injected into the nucleus of an animal cell, using a microsyringe. This is known as **microinjection**.
- Plant cells are bombarded with high velocity microparticles of gold or tungsten coated with DNA in a method known as **biolistics** or **gene gun**.
- Some pathogens that naturally infect a cell can be 'disarmed' (by eliminating their harmful gene) and then allowed to infect the cell, carrying the desired recombinant DNA into the host.

Processes of Recombinant DNA Technology

- Recombinant DNA technology involves various steps in a specific sequence such as isolation of the desired genetic material (DNA), cutting of DNA at specific locations, isolation of desired DNA fragment, amplification of gene of interest by PCR, ligation of DNA fragments into a vector, insertion of recombinant DNA into host cell, culturing the host cells in a medium at large scale and interaction of the desired product.

Isolation of the Genetic Material (DNA)

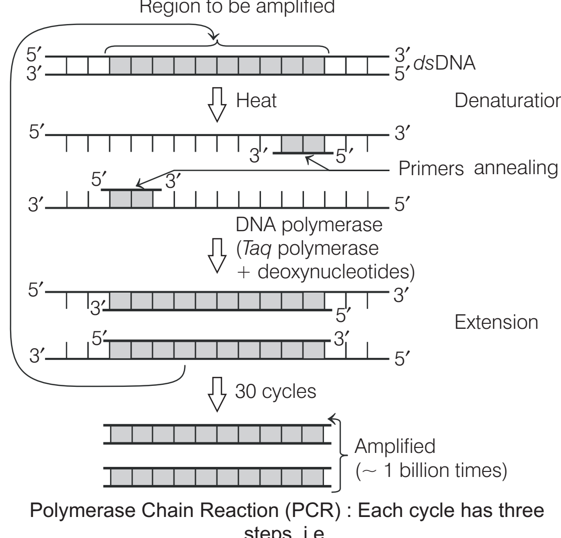
- The genetic material, DNA needs to be in pure form, free from other macromolecules, for restriction enzymes to be able to act on it. Following are the steps
  - Cell wall needs to be broken to release DNA. For this, the bacterial cells/plant or animal tissue are treated with enzymes such as **lysozyme** (bacteria), **cellulase** (plant cells), **chitinase** (fungus).
  - Histones and other proteins have to be removed by treatment with proteases and the RNA can be removed by treatment with ribonuclease.
  - With the addition of chilled ethanol, purified DNA precipitates out. This can be seen as collection of fine threads in the suspension and removed out by spooling.

Amplification of Gene of Interest Using PCR

- **PCR** stands for **Polymerase Chain Reaction**, a method of amplifying the fragments of DNA. This method can make multiple copies of even a single DNA fragment or the gene of interest in a test tube. The reaction mixture requires
  - Double-stranded DNA fragment (**gene of interest**).
  - **Primers**, i.e. small chemially synthesised oligonucleotides that are complementary to the regions of this DNA.

- The special thermostable **DNA polymerase** (isolated from the bacterium, *Thermus aquaticus*) that does not denature and remains active even at high temperatures.

- With each round of reaction, the DNA quantity gets amplified. If the process of replication of DNA is repeated many times the segment of DNA can be amplified to approximately billion times, i.e. 1 billion copies are made.



Polymerase Chain Reaction (PCR) : Each cycle has three steps, i.e.

Insertion of Recombinant DNA into the Host Cell/Organism

- There are several methods of introducing the ligated DNA into recipient cells. Recipient cells after making them competent to receive take up DNA present in their surrounding.
- So, if a recombinant DNA bearing gene for resistance to an antibiotic (e.g. ampicillin) is transferred into *E. coli* cells, the host cells are transformed into ampicillin-resistant cells.
- If we spread the transformed cells on agar plates containing ampicillin, only transformants will grow and untransformed recipient cells will die.
- Since, due to ampicillin resistance gene, one is able to select a transformed cell in the presence of ampicillin. The ampicillin resistance gene in this case is called a **selectable marker**.

Obtaining the Foreign Gene Product

- The foreign gene gets expressed under appropriate conditions. After having the cloned gene of interest and having optimised conditions to induce the expression of the target protein, large scale production can be carried out.
- If any protein encoding gene is expressed in a heterologous host, the product protein is called a **recombinant protein**.
- The cells harbouring cloned genes of interest may be grown on a small scale in the laboratory. The cultures may be used for extracting the desired protein. The cells can also be multiplied in a continuous culture system, wherein the used medium is drained out from one side, while fresh medium is added from the other to maintain the cells in their physiologically most active growth phase, i.e. log/exponential phase. This type of culturing method produces a larger biomass leading to higher yields of desired protein. For large scale production, bioreactors are used and culture can be processed.
- **Bioreactors** are large vessels (100-1000 L capacity) in which raw materials are biologically converted into specific products, individual enzymes, etc., using microbial, plant, animal or human cells.
  These are specially designed to provide optimal conditions of temperature, pH, substrate, salts, vitamins, oxygen, etc., for achieving the desired production levels.
- Bioreactors can be of two types–**continuous culture system** and **non-continuous culture system** (Batch culture). The two variants of bioreactors are sparged tank bioreactor and simple stirred-tank bioreactor. A stirred tank reactor is usually cylindrical or with a curved base to facilitate the even mixing and oxygen availability throughout the bioreactor. It is the most commonly used type of bioreactor.

Downstream Processing

The steps of downstream processing are separation and purification of the gene product, i.e. the functional protein, formulating it with suitable formulation. Such formulations undergo clinical trials to determine product quality. The downstream processing and quality control testing vary from product to product.

Mastering NCERT

MULTIPLE CHOICE QUESTIONS

TOPIC 1 ~ *Principles of Biotechnology and Restriction Enzymes*

1 The controlled use of biological agents, such as live organisms or enzymes from organisms to produce products and processes useful to humans is called as
(a) biochemistry (b) molecular biology
(c) biotechnology (d) microbiology

2 The techniques/processes that are included under biotechnology are
(a) *in vitro* fertilisation (b) correcting a defective gene
(c) synthesising a gene (d) All of these

3 EFB stands for
(a) European Federation of Biotechnology
(b) Eurasian Federation of Biotechnology
(c) East Asia Federation of Biotechnology
(d) Ethiopian Federation of Biotechnology

4 The definition of biotechnology given by EFB encompasses
(a) traditional biotechnology
(b) modern molecular biotechnology
(c) DNA fingerprinting
(d) Both (a) and (b)

5 The two main techniques that gave birth to modern biotechnology are
I. bioprocess engineering.
II. genetic engineering.
III. human genome engineering.
IV. molecular biology.
Choose the correct option.
(a) I and II (b) I and III (c) II and IV (d) II and III

6 Genetic engineering techniques include
(a) altering genetic material
(b) sequencing genetic material
(c) studying genetic material
(d) None of the above

7 The specific sequence of DNA that initiate replication of alien DNA in *r*DNA technology is called as
(a) initiation sequence (b) origin of replication
(c) origin of DNA (d) initiation of DNA

8 Autonomously replicating circular extra-chromosomal DNA is
(a) vector (b) capsid
(c) plasmid (d) bacteriophage

9 The construction of the first recombinant DNA was done by using the native plasmid of
(a) *E. coli* (b) *Salmonella typhimurium*
(c) *Bacillus thuringiensis* (d) Yeast

10 The first recombinant DNA was constructed by
(a) Stanley Cohen (b) Herbert Boyer
(c) Temin and Baltimore (d) Both (a) and (b)

11 The linking of antibiotic resistance gene with the plasmid vector became possible with
(a) DNA ligase (b) RNA ligase
(c) DNA polymerase (d) RNA polymerase

12 The different basic steps of genetically modifying an organism are given below randomly.
I. Identification of DNA with desirable genes.
II. Transfer of the DNA to its progeny.
III. Maintenance of introduced DNA in the host.
IV. Introduction of identified DNA into the host.
Which of the following represents the correct sequence of steps?
(a) I, II, III and IV (b) I, IV, III and II
(c) III, IV, II and I (d) I, III, IV and II

13 The key tools required for the recombinant DNA technology are
I. restriction enzymes II. polymerase enzymes
III. ligases IV. vector
V. host organism
Select the correct option.
(a) I, II and III (b) I, III, IV and V
(c) I, II, III and V (d) I, II, III, IV and V

14 The enzymes, commonly used in genetic engineering are
(a) restriction endonuclease and polymerase
(b) endonuclease and ligase
(c) restriction endonuclease and ligase
(d) ligase and polymerase

15 The first restriction endonuclease to be discovered is
(a) *Hin*d III (b) *Hin*d II (c) *Eco* RI (d) *Eco* RII

16 Which of the following is a restriction endonuclease? **CBSE-AIPMT 2015**
(a) Protease (b) DNase I (c) RNase (d) *Hin*d II

17 The restriction enzyme responsible for the cleavage of following sequence is

$$5' - G\ T\ C\ G\ A\ C\ 3'$$
$$3' - C\ A\ G\ C\ T\ G\ 5'$$

(a) *Alu* I (b) *Bam* HI (c) *Hin*d II (d) *Eco* RI

18 How many restriction enzymes are isolated till now?
(a) 920 (b) 940 (c) 900 (d) 230

19 Number of bacterial strains from which restriction enzymes have been isolated.
(a) 230 (b) 250 (c) 200 (d) 220

20 In the naming of restriction enzymes, the first letter of the name is derived from ..... *A* .... and next two letters from the ..... *B* ..... and fourth letters from the name of ..... *C* .... of .... *D* .... from which the enzymes are extracted.
A to *D* in the statement can be

	A	B	C	D
(a)	genus	species	strain	bacteria
(b)	species	genus	strain	bacteria
(c)	genus	species	variety	eukaryote
(d)	species	genus	variety	eukaryote

21 There is a restriction endonuclease called *Eco* RI. What does '*co*' part in it stands for?
(a) Coelom (b) Strain of bacterium
(c) *coli* (d) Colon

22 The Roman number following the name of restriction enzyme indicate
(a) order in which enzyme is isolated from strain of bacteria
(b) number of enzyme
(c) order of enzyme
(d) None of the above

23 Restriction enzyme belongs to which class of enzymes?
(a) Ligases (b) Exonucleases
(c) Nucleases (d) Proteases

24 In a genetic engineering experiment, restriction enzymes can be used for
(a) bacterial DNA only (b) viral DNA only
(c) any DNA fragment (d) eukaryotic DNA only

25 An enzyme catalysing the removal of nucleotides from ends of DNA is ***NEET (Odisha) 2019***
(a) DNA ligase (b) endonuclease
(c) exonuclease (d) protease

26 Restriction endonucleases are enzymes which
(a) make cuts at any position within the DNA molecule
(b) recognise a specific nucleotide sequence for binding and then cleaves both the strands of DNA
(c) restrict the action of the enzyme DNA polymerase
(d) remove nucleotides from the ends of the DNA molecule

27 Restriction endonuclease enzymes are used to cut
(a) single-stranded RNA (b) double-stranded DNA
(c) single-stranded DNA (d) double-stranded RNA

28 Restriction endonuclease binds to DNA and cuts two strands of double helix at specific points in their
(a) sugar-phosphate backbone
(b) hydrogen bond
(c) glycosidic bonds
(d) None of the above

29 Special sequence in the DNA recognised by restriction endonuclease is called
(a) restriction nucleotide sequence
(b) palindromic nucleotide sequence
(c) recognition nucleotide sequence
(d) All of the above

30 Palindrome sequences
(a) read opposite on two strands
(b) read specific sequence in opposite direction
(c) read same on two strands when orientation of reading is same
(d) read opposite on two strands when orientation of reading is same

31 Restriction enzyme cuts the DNA strand a little away from the centre of palindrome site between
(a) same two bases on same strand
(b) same two bases on opposite strand
(c) opposite bases on same strand
(d) opposite bases on opposite strand

32 The foreign DNA and the vector is cut with the
(a) two different enzymes
(b) same restriction enzymes
(c) DNA ligase
(d) Both (a) and (b)

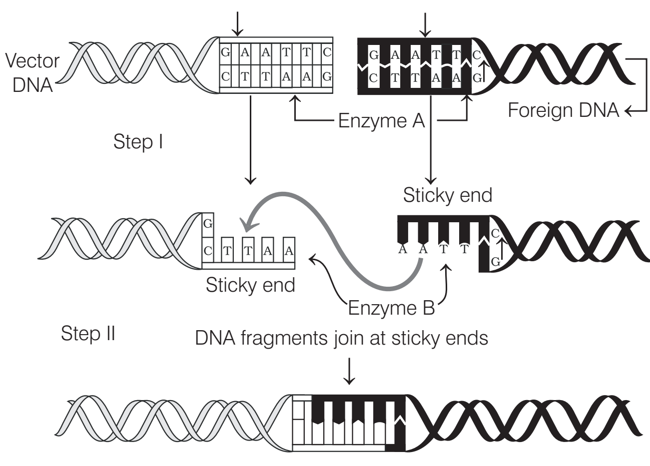
33 How many fragments will be generated, if a linear DNA molecule is digested with a restriction enzyme having 4 recognition sites on the DNA?
(a) 3 (b) 5
(c) 4 (d) 6

34 How many fragments will be generated, if a closed circular DNA molecule is digested using a restriction enzyme having six recognition sites on the DNA?
(a) 4 (b) 6 (c) 7 (d) 5

35 A foreign DNA and plasmid cut by the same restriction endouclease can be joined to form a recombinant plasmid using ***NEET 2016***
(a) *Eco* RI (b) *Taq* polymerase
(c) polymerase III (d) ligase

36 Which is also called molecular glue? ***JIPMER 2019***
(a) DNA gyrase (b) DNA helicase
(c) DNA ligase (d) DNA polymerase

37 Study the given diagram and identify the enzymes *A* and *B* involved in steps I and II.

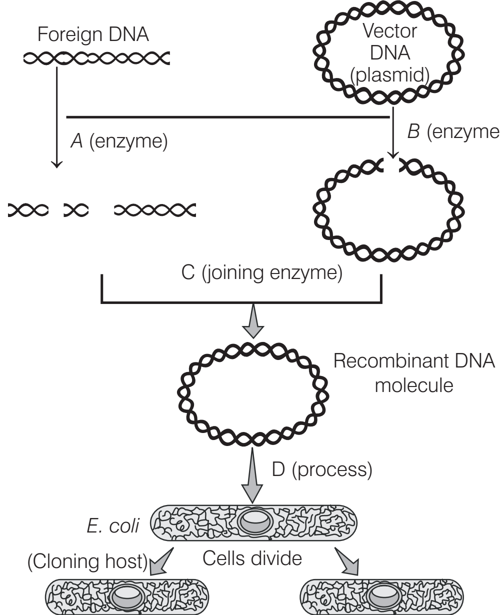


Step I	Step II
(a) *Eco* RI	DNA ligase
(b) *Alu* I	DNA ligase
(c) *Hin*d II	DNA polymerase
(d) Restriction endonuclease	DNA polymerase

38 Which of the following option (s) is not correct regarding *Eco* RI enzyme?

(a) Restriction endonuclease enzyme
(b) Isolated from *Escherichia coli* RY13
(c) Cuts at specific position within the DNA
(d) None of the above

39 The flowchart given below represents the process of recombinant technology. Identify *A* to *D* in the given process.



(a) A–Restriction endonuclease, B–Restriction exonuclease, C–RNA ligase, D–Transformation
(b) A–Restriction endonuclease, B–Restriction endonuclease, C–DNA ligase, D–Transformation
(c) A–Restriction exonuclease, B–Restriction endonuclease, C–DNA polymerase, D–Transduction
(d) A–Restriction endonuclease, B–Restriction endonuclease, C–DNA polymerase, D–Transformation

40 The cutting of DNA by ……… results in the fragments of DNA. Choose the appropriate option.

(a) restriction endonucleases
(b) exonuclease
(c) endonuclease
(d) anhydro L-galactose

41 DNA fragments generated by the restriction endonucleases in a chemical reaction can be separated by ***NEET 2013***

(a) centrifugation
(b) polymerase chain reaction
(c) electrophoresis
(d) restriction mapping

42 Which of the following techniques is most commonly used to separate DNA molecules by size?

(a) Chromatography (b) PCR
(c) RFLP (d) Gel electrophoresis

43 Agarose is extracted from

(a) sea weeds (b) blue-green algae
(c) *Ephedra* (d) *Sargassam*

44 What is the criterion for DNA fragments movements on agarose gel during gel electrophoresis ? ***NEET 2017***

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

45 In gel electrophoresis, restriction enzyme digested DNA is loaded in wells near

(a) anode (b) cathode
(c) centre of gel (d) any where in the gel

46 Having become an expert on gel electrophoresis, you are asked to examine a gel for a colleague. Where would you find the smallest fragments of DNA?

(a) Near the positive electrode, farthest away from the wells
(b) Near the negative electrode, close to the wells
(c) Near the top, near the negative pole
(d) Near the middle they tend to slow-down after the first few minutes

47 The DNA fragments separated on an agarose gel can be visualised after staining with ***NEET 2017***

(a) bromophenol blue
(b) acetocarmins
(c) aniline blue
(d) ethidium bromide

48 In gel electrophoresis, the separated DNA fragments are visualised after staining the DNA with EtBr followed by exposure to …. .

Choose the appropriate option.

(a) Infrared radiation (b) UV-radiation
(c) γ-rays (d) Radiowave

49 When the DNA fragments are observed under UV light, they are seen as

(a) yellow coloured bands
(b) orange coloured bands
(c) blue coloured bands
(d) Both (a) and (b)

50 In gel electrophoresis, the separated bands of DNA are cut out and extracted from the gel piece. This step is called

(a) elution
(b) origin of replication
(c) competency
(d) transformation

TOPIC 2 ~ *Cloning Vectors and Competent Host*

51 In recombinant DNA technique, the term vector refers to a

(a) donor DNA, it is identified and picked up through electrophoresis
(b) plasmid transfers DNA into host cell
(c) collection of entire genome in the form of plasmid
(d) enzyme, cuts the DNA at specific sites

52 Which of the following is used in recombinant DNA technique?

(a) Cell wall of virus
(b) Gene which produces capsid of virus
(c) Bacteriophage
(d) Capsid of virus

53 During 'gene cloning' which is called a gene taxi?

(a) Vaccine (b) Plasmid
(c) Bacteria (d) Protozoa

54 Which of the following is not a feature of the plasmids? ***CBSE-AIPMT 2015***

(a) Circular structure
(b) Transferable
(c) Single-stranded
(d) Independent replication

55 Which vector can clone only a small fragment of DNA? ***CBSE-AIPMT 2014***

(a) Bacterial artificial chromosome
(b) Yeast artificial chromosome
(c) Plasmid
(d) Cosmid

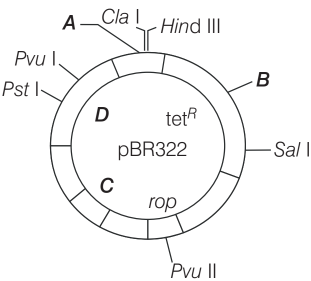
56 The DNA used as a carrier for transferring a fragment of foreign DNA into a suitable host is called

(a) cloning vector (b) vehicle DNA
(c) gene carrier (d) All of these

57 Which of the following is a plasmid vector?

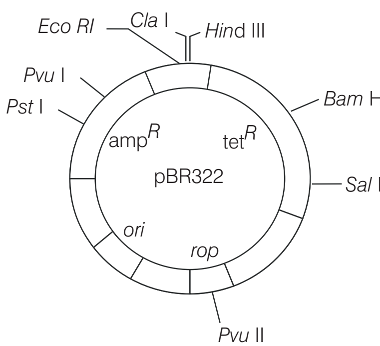
(a) pBR322 (b) *Bam* II
(c) *SaI* I (d) *Eco* RI

58 Identify *A, B, C* and *D* in the given diagram of *E. coli* cloning vector pBR322.



(a) A–*Eco* RI, B–*Bam* HI, C–*ori*, D–amp^R
(b) A–amp^R, B–*ori*, C–*Bam* HI, D–*Eco* RI
(c) A–*ori*, B–*Bam* HI, C–*Eco* RI, D–amp^R
(d) A–*Bam* HI, B–*Eco* RI, C–amp^R, D–*ori*

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



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

60 The two antibiotic resistance genes on vector pBR322 are for ***NEET (Odisha) 2019***
(a) ampicillin and tetracycline
(b) ampicillin and chloramphenicol
(c) chloramphenicol and tetracycline
(d) tetracycline and kanamycin

61 The function of *ori* in a vector is
(a) help in replication of linked DNA
(b) control copy number of the linked DNA
(c) help in selecting recombinants
(d) Both (a) and (b)

62 A gene, whose expression helps to identify transformed cells is known as ***NEET 2017***
(a) selectable marker (b) vector
(c) plasmid (d) structural gene

63 A selectable marker is used to ***NEET (Odisha) 2019***
(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 tansformation in a specific crop
(d) mark a gene on a chromosome for isolation using restriction enzyme

64 If recombinant DNA carrying antibiotic resistance gene (e.g. ampicillin) is transferred into *E. coli* cell, the host cell is transformed into ampicillin resistant cells. The ampicillin resistant gene in this case is called a
(a) vectors (b) plasmid
(c) selectable marker (d) cloning sites

65 The presence of more than one recognition site within vector will lead to the
(a) generation of several fragments
(b) generation of one fragment
(c) generation of half fragment
(d) None of the above

66 The recognition site for *Bam* HI in pBR322 is present in
(a) ampicillin resistant site (b) tetracycline resistant site
(c) *ori* site (d) *rop* site

67 When an alien DNA is ligated in tetracycline resistant gene, the recombinant
(a) become tetracycline resistant
(b) will loose tetracycline resistant
(c) will remain same
(d) None of the above

68 The method(s) that is/are used to differentiate recombinants and non-recombinants is/are
(a) antibiotic affected gene (b) insertional inactivation
(c) gene cloning (d) Both (a) and (b)

69 In insertional inactivation, the recombinant DNA is inserted within the coding sequence of
(a) β-galactosidase (b) tetracycline resistant gene
(c) restriction enzyme (d) ampicillin resistant gene

70 Recombinant colonies in insertional inactivation are differentiated on the basis of
(a) production of blue colour
(b) production of no colour
(c) production of red colour
(d) production of green colour

71 *Agrobacterium tumefaciens* delivers a piece of DNA into dicot plant. The piece of DNA is called as
(a) *r*DNA (b) T-DNA (c) *m*DNA (d) *c*DNA

72 Retroviruses in animals including humans are able to change normal cells into
(a) germ cell (b) cancerous cells
(c) cosmid (d) vector

73 The plasmid of *Agrobacterium tumefaciens* that is now modified as a cloning vector is ***AIIMS 2019***
(a) Pi-plasmid (b) cosmid
(c) Ti-plasmid (d) None of these

74 In Ti-plasmid, which of the following is removed? ***AIIMS 2019***
(a) Auxin gene (b) Virulent gene
(c) Cytokinin gene (d) Auxin and cytokinin gene

75 Why foreign DNA cannot pass through cell membrane?
(a) DNA is hydrophobic
(b) DNA is hydrophilic
(c) DNA is rich in proteins
(d) DNA is heavy

76 The treatment of host cell with divalent cation leads to the
(a) change in permeability of DNA
(b) increased efficiency with which DNA enters the bacterium
(c) decreased efficiency with which DNA enters the bacterium
(d) change in permeability of host

77 The method which is used to introduce recombinant DNA into animal cell?
(a) Gene gun method (b) Changing permeability of host
(c) Biolistic method (d) Microinjection

78 Which of the following methods(s) is used to introduce foreign DNA into plant host cells?
(a) Gene gun method (b) Gel electrophoresis
(c) Elution (d) Extension

79 For transformation, microparticles coated with DNA to be bombarded with gene gun are made up of ***CBSE-AIPMT 2012***
(a) silver or platinum (b) platinum or zinc
(c) silicon or platinum (d) gold or tungsten

80 DNA transfer with high velocity micro particles is present in ***JIPMER 2018***
(a) biolistics (b) hybridisation
(c) tissue culture (d) vegetative propagation

TOPIC 3 ~ Processes of Recombinant DNA Technology

81 The different steps involved in the process of recombinant DNA technology are given below randomly? Arrange these in correct order.

I. Extraction of the desired gene product.
II. Amplification of the gene of interest.
III. Isolation of a desired DNA fragment.
IV. Ligation of the DNA fragment into a vector.
V. Insertion of recombinant DNA into the host.

Correct order is

(a) I, II, III, IV and V (b) III, II, IV, V and I
(c) II, IV, V, III and I (d) I, IV, V, III and II

82 In bacterial cells, the membrane is digested with the help of enzyme

(a) cellulase (b) lysozyme
(c) chitinase (d) lipase

83 RNA is removed by the treatment with

(a) ribonuclease (b) protease
(c) chitinase (d) cellulase

84 Proteins are removed by treatment with

(a) ribonuclease (b) chitinase
(c) cellulase (d) protease

85 DNA precipitation out of a mixture of biomolecules can be achieved by treatment with ***NEET 2019***

(a) chilled ethanol
(b) methanol at room temperature
(c) chilled chloroform
(d) isopropanol

86 Purified DNA ultimately precipitates out and this can be seen as collection of fine threads in the suspension as seen in the figure. It refers to

(a) DNA Spooling (b) DNA digestion
(c) DNA recognition (d) DNA bands

87 Chimeric DNA is ***AIIMS 2019***

(a) gene clone (b) recombinant DNA
(c) transposon (d) vector shuttle

88 Polymerase Chain Reaction (PCR) needs

(a) DNA template (b) Primers
(c) *Taq* polymerase (d) All of these

89 Primers are

(a) small chemically synthesised oligonucleotides of about 10-18 nucleotides that are complementary to the region of template DNA
(b) chemically synthesised oligonucleotides of about 10-18 nucleotides that are not complementary to the region of template DNA
(c) the double-stranded DNA that need to be amplified
(d) specific sequences present on recombinant DNA

90 The *Taq* polymerase enzyme is obtained from ***CBSE-AIPMT 2015***

(a) *Thiobacillus ferroxidans*
(b) *Bacillus subtilis*
(c) *Pseudomonas subtilis*
(d) *Thermus aquaticus*

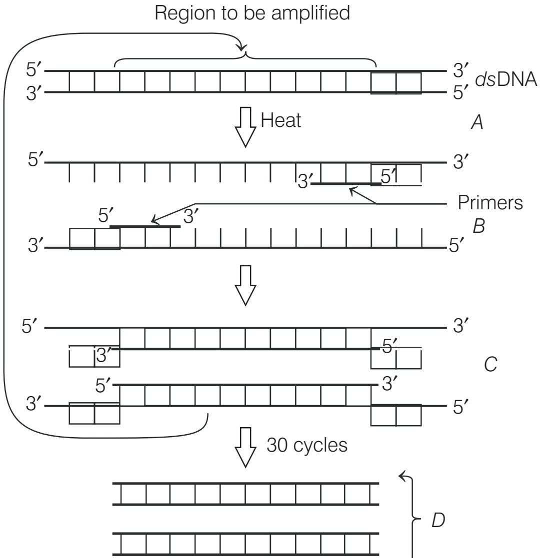
91 A single PCR amplification cycle involves

(a) denaturation (b) extension
(c) annealing (d) All of these

92 The correct order of steps in Polymerase Chain Reaction (PCR) is ***NEET 2018***

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

93 The below diagram refer to PCR. Identify the steps *A*, *B*, *C* and *D*. Select the correct option.



(a) A–Denaturation at 94-96°C, B–Annealing at 40-60°C, C–Extension through *Taq* polymerase at 72°C, D–Amplified

(b) A–Annealing at 94-96°C, B–Denaturation at 40-60°C, C–Extension through *Taq* polymerase at 72°C, D–Amplification
(c) A–Extension through *Taq* polymerase at 40-60°C, B–Amplification, C–Denaturation at 40-60°C, D–Annealing at 94-96°C
(d) A–Amplification, B–Extension through *Taq* polymerase at 40-60°C, C–Denaturation at 40-60°C, D–Annealing at 94-96°C

94 If a recombinant DNA bearing gene for ampicllin resitance is transferred into *E. coli* and the host cells are spread on agar plates containing ampicillin, then ***AIIMS 2018***

(a) both transformed and untransformed recipient cells will die
(b) both transformed and untransformed recipient cells will grow
(c) transformed recipient cells will grow and untransformed recipient cells will die
(d) transformed recipient cells will die and untransformed recipient cells will grow

95 Protein encoding gene which is expressed in heterologous host is
(a) foreign protein (b) heterologous protein
(c) recombinant protein (d) alien protein

TOPIC 4 ~ *Bioreactors and Downstream Processing*

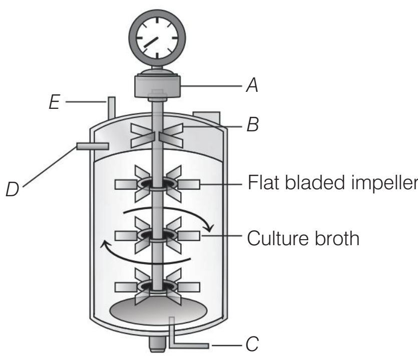
96 In continuous culture system,
(a) used medium is drained out
(b) biomass produced is high
(c) no new medium is added
(d) Both (a) and (b)

97 A bioreactor
(a) is hybridoma
(b) cultures products containing radioactive isotopes
(c) cultures for the synthesis of new chemicals
(d) cultures large volume of living cells

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

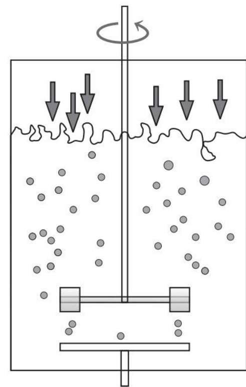
99 Stirred-tank bioreactors are advantageous over shake flasks because they
(a) provide high temperature and pH
(b) provide better aeration and mixing properties
(c) do not allow the entry of CO_2
(d) are easy to operate

100 Simple stirred-tank bioreactor is given below. Identify *A, B, C, D* and *E*.



	A	B	C	D	E
(a)	Motor	Foam breaker	Sterile air	Steam for sterilisation	Acid/base for pH control
(b)	Foam breaker	Sterile air	Steam for sterilisation	Acid/Base for pH control	Motor
(c)	Acid/base for pH control	Motor	Foam breaker	Sterile air	Steam for sterilisation
(d)	Sterile air	Steam for sterilisation	Foam breaker	Motor	Acid/base for pH control

101 Identify the correct match for the given apparatus.



	Apparatus	Functions
(a)	Gene gun	Vectorless direct gene transfer
(b)	Column chromatograph	Separation of chlorophyll pigments
(c)	Sparged tank bioreactor	Carry out fermentation process
(d)	Respirometer	Finding out rate of respiration

102 The components of a bioreactor are

I. an agitator system.
II. an oxygen delivery system.
III. foam control system.
IV. temperature control system.
V. pH control system.
VI. sampling ports to withdraw cultures periodically.

Choose the correct option.

(a) I, II, III, IV and V (b) II, IV, V and VI
(c) I, II, III, IV and VI (d) All of these

103 The process of separation and purification of expressed protein before marketing is called *NEET 2017*

(a) upstream processing
(b) downstream processing
(c) bioprocessing
(d) post-production processing

104 Which of the following is not a component of downstream processing? *NEET 2016*

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

NEET

SPECIAL TYPES QUESTIONS

I. Assertion and Reason

■ **Direction** (Q. No. 105-113) *In each of the following questions, a statement of Assertion (A) is given followed by corresponding statement of Reason (R). Of the statements, mark the correct answer as*

(a) If both A and R are true and R is the correct explanation of A
(b) If both A and R are true, but R is not the correct explanation of A
(c) If A is true, but R is false
(d) If A is false, but R is true

105 **Assertion** (A) Biotechnology deals with techniques that use living organism to produce products useful for humans.

Reason (R) It uses only a unicellular organism.

106 **Assertion** (A) Maintenance of sterile environment is essential for manufacture of biotechnological products.

Reason (R) This is to enable growth of desired prokaryotic or eukaryotic cells.

107 **Assertion** (A) Origin of replication is an essential part of a vector.

Reason (R) *Ori* is responsible for initiating replication.

108 **Assertion** (A) Foreign DNA and vector DNA cut with the help of ligase.

Reason (R) Ligase acts by forming phosphodiester bonds.

109 **Assertion** (A) In gel electrophoresis, DNA fragments are separated.

Reason (R) DNA is negatively charged, so it moves towards anode under electric field.

110 **Assertion** (A) Restriction endonucleases are also called 'molecular scissors'.

Reason (R) When fragments generated by restriction endonucleases are mixed, they join together due to their sticky ends.

111 **Assertion** (A) All endonucleases cut DNA at specific sites.

Reason (R) Endonucleases were discovered from viruses.

112 **Assertion** (A) The tumour inducing plasmid (Ti plasmid) of *Agrobacterium tumefaciens* acts as a cloning vector in recombinant DNA technology.

Reason (R) The Ti plasmid which is used in the mechanisms of delivering genes to a cell remains pathogenic.

113 **Assertion** (A) Use of chitinase enzyme is necessary for isolation of DNA from fungal cells.

Reason (R) Fungal cell wall is made up of chitin and chitinase is able to digest it.

II. Statement Based Questions

114 Which one is a true statement regarding DNA polymerase used in PCR? *CBSE-AIPMT 2012*

(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

115 Following statements describe the characteristics of the enzyme restriction endonulease. Identify the incorrect statement. *NEET 2019*

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

(b) The enzyme cuts the sugar-phosphate backbone at specific sites on each strand
(c) The enzyme recognises a specific palindromic nucleotide sequence in the DNA
(d) The enzyme cuts DNA molecules at identified position within the DNA

116 Which of the following statement is incorrect?
(a) Nucleic acid is fragmented by nucleases
(b) Construction of recombinant DNA involves cleaning DNA segments with endonuclease and rejoining with ligase
(c) Genetic engineering is making artificial limbs and diagnostic instruments
(d) Ti plasmid transforms cells of plants

117 Which of the following statement is incorrect?
(a) DNA being a hydrophilic molecule cannot pass through cell membranes
(b) *Agrobacterium tumefaciens* delivers a piece of DNA known as 'Z-DNA' which transforms normal plant cells into tumour cells and directs these tumour cells to produce chemicals against pathogens
(c) Retrovirus, adenovirus, papillomavirus are also now used as cloning vectors in animal because of their ability to transform normal cells into cancerous cell
(d) In genetic engineering, DNA from different sources are cut with the same restriction enzymes so that both DNA fragments have same kind of sticky ends

118 Consider the following statements and select the correct option.
(a) A soil inhabiting plant bacterium, *Agrobacterium tumefaciens*, a pathogen of several dicot plants is able to transfer a piece of DNA known as T-DNA
(b) The T-DNA causes tumours
(c) Tumour formation is induced by Ti plasmid
(d) All of the above

119 Which of the following statement is incorrect?
(a) Each restriction endonuclease recognises a specific palindromic nucleotide sequence
(b) Specific base sequence is known as recognition sequence
(c) Restriction enzymes cannot cut DNA
(d) Restriction enzymes belong to enzymes called nucleases

120 Which of the following statement is incorrect?
(a) *Eco*RI cuts the DNA between bases G and A
(b) Making multiple identical copies of any template DNA is called cloning
(c) pBR322 is a natural plasmid
(d) *Agrobacterium tumefaciens* is a natural genetic engineer

121 Choose the incorrect statement.
(a) *Ori* also controls the copy numbers of the linked DNA
(b) If a foreign DNA ligates at the *Bam* HI site of tetracycline resistance gene in the vector pBR322, the recombinant plasmid loses the tetracycline resistance due to insertion of foreign DNA
(c) Copy number refers to the number of copies of plasmid present in a cell
(d) Copy number of plasmid varies from 50-100 per cell

122 Which of the statements given is incorrect?
(a) In microinjection method, foreign DNA is directly injected into the nucleus of animal cell by using microneedles
(b) Microinjection method is used in oocytes, eggs and embryo
(c) Electroporation is the formation of temporary pores in the plasma membrane of host cell by using lysozyme or calcium chloride
(d) In chemical mediated gene transfer method, certain chemicals such as Ca phosphate help foreign DNA to enter the host cell

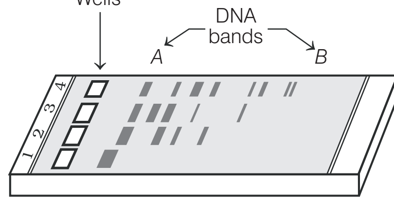
123 Consider the following statements.
I. Recombinant DNA technology popularly known as genetic engineering is a stream of biotechnology which deals with the manipulation of genetic material by man *in vitro*.
II. pBR322 is the first artificial cloning vector developed in 1977 by Boliver and Rodriquez from *E. coli* plasmid.
III. Restriction enzymes belong to a class of enzymes called nucleases.

Which of the statements given above are correct?
(a) I and II (b) I and III (c) II and III (d) I, II and III

124 Given below are four statements pertaining to separation of DNA fragments using gel electrophoresis. Identify the incorrect statements.
NEET (Odisha) 2019
I. DNA is negatively charged molecule and so it is loaded on gel towards the anode terminal.
II. DNA fragments travel along the surface of the gel whose concentration does not affect movement of DNA.
III. Smaller the size of DNA fragment larger is the distance it travels through it.
IV. Pure DNA can be visualised directly by exposing UV-radiation.

Select the correct option from the following.
(a) I, III and IV (b) I, II and III
(c) II, III and IV (d) I, II and IV

125 Study the given figure carefully and select the correct statements regarding this.



I. It represents typical agarose gel electrophoresis showing differential migration of DNA fragments.
II. Lane 1 contains undigested DNA fragments.
III. Lanes 2 to 4 contain digested DNA fragment.
IV. Smallest DNA bands are present at *A* position and largest DNA bands are present at *B* position.

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

126 Read the statements about gene gun method.
I. This method is also known as biolistic technique.
II. In this method, cells are bombarded with high velocity microparticles of gold or tungsten coated with DNA in plants.
III. Important crop plants like maize, rice and wheat have now been transformed by this method.

Which of the statements given above are correct?
(a) I and II (b) I and III (c) II and III (d) I, II and III

127 Identify the correct statements.
I. The first recombinant DNA was constructed by using a piece of DNA from plasmid carrying antibiotic-resistance gene in the bacterium *Salmonella typhimurium* and linked it to the plasmid of *E. coli*.
II. When cut by the same restriction enzyme, the resultant DNA fragments have the same kind of sticky ends and these can be joined together using DNA ligases.
III. The presence of more than one recognition sites within the vector will generate several fragments, which will complicate the gene cloning.

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

128 Read the statements.
I. In the process of recombinant DNA technology, after several treatment the purified DNA is precipitated by adding chilled acetone.
II. The bacterial/plant, animal cell is broken down by enzymes to release DNA, along with RNA, proteins, polysaccharides and lipids.

Choose the correct option for above statements.
(a) I is true, but II is false (b) I is false, but II is true
(c) I and II are true (d) I and II are false

129 Which of the following statements are correct with respect to a bioreactor?
I. It can process small volume of culture.
II. It provides optimum temperature, pH, salt, vitamins and oxygen.
III. Sparged stirred-tank bioreactor is a stirred type reactor in which air is bubbled.

Choose the correct option.
(a) I and II (b) I and III
(c) II and III (d) I, II and III

130 Consider the following statements.
I. Bioreactors are vessels of large volumes in which raw materials are biologically converted into specific products.
II. One of the most commonly used bioreactor is of stirring type.
III. Shake flasks are used for growing and mixing the desired materials on a small scale in the laboratory.
IV. A large scale production of desired biotechnological product is done by using ‘bioreactors’.

Which of the statements given above are correct?
(a) I and II (b) I and III
(c) I, II and III (d) I, II, III and IV

131 Which statement is correct?
I. The downstream processing and quality control testing vary from product to product.
II. In bioreactors, raw materials are biologically converted into specific products.
III. Large amount of recombinant protein can be produced by gene cloning.
IV. pBR322 vector was constructed by using DNA derived from naturally occurring plasmids of *E. coli*.

(a) I, II and III (b) Only IV
(c) II, III and IV (d) All of these

132 Which statement is incorrect ?
I. Retroviruses have also been disarmed and are now used to deliver desirable genes into animals cells.
II. Downstream processing is one of the steps of R-DNA technology.
III. DNA is a negatively charged molecule.
IV. The presence of chromogenic substrate gives blue colour colonies, if the plasmid in the bacteria does not have an insert.

(a) I and II (b) I, III and IV
(c) All of these (d) None of these

133 For selectable marker.
I. It helps to select the host cells which contain the vector and eliminate the non-transformants.
II. Genes encoding resistance to antibiotics like ampicillin, chloramphenicol, tetracycline or kanamycin, are useful selectable markers for *E.coli*.

Which of the statements given above are correct?
(a) Only I (b) Only II
(c) I and II (d) None of these

134 I. DNA being a hydrophilic molecule cannot pass through cell membranes.
II. The bacteria should be made competent to accept the DNA molecule.

The correct option regarding the above statements is
(a) I is true, but II is false (b) II is true, but I is false
(c) I and II are true (d) I and II are false

III. Matching Type Questions

135 Match the following columns.

	Column I		Column II
A.	Recombinant DNA	1.	Sea weeds
B.	Gel electrophoresis	2.	DNA staining
C.	Ethidium bromide	3.	Plasmid DNA that has incorporated human DNA
D.	Agarose	4.	Process by which DNA fragments are separated based on their size

Codes

	A	B	C	D		A	B	C	D
(a)	3	4	2	1	(b)	3	2	1	4
(c)	2	1	4	3	(d)	3	4	1	2

136 Match the Column I with Column II with respect to the nomenclature of enzyme *Eco* RI and select the correct answer from codes given below.

	Column I		Column II
A.	*E*	1.	Ist in order of identification
B.	*co*	2.	genus
C.	R	3.	species
D.	I	4.	strain

Codes

	A	B	C	D		A	B	C	D
(a)	3	4	1	2	(b)	2	3	4	1
(c)	2	1	4	3	(d)	2	3	1	4

137 Match the following columns.

	Column I		Column II
A.	Bacterial cell is treated with	1.	Lysozyme
B.	Plant cell is treated with	2.	Cellulase
C.	Fungal cell is treated with	3.	Chitinase

Codes

	A	B	C		A	B	C
(a)	3	2	1	(b)	2	3	1
(c)	1	2	3	(d)	3	1	2

138 Match the following columns.

	Column I		Column II
A.	Isolation of purified DNA	1.	Restriction enzyme
B.	Cutting of DNA at specific location	2.	Gel electrophoresis
C.	Isolation of DNA fragments	3.	Chilled ethanol
D.	Amplification of gene	4.	PCR

Codes

	A	B	C	D		A	B	C	D
(a)	1	2	3	4	(b)	3	1	2	4
(c)	2	1	3	4	(d)	3	2	1	4

139 Match the following columns.

	Column I		Column II
A.	PCR	1.	Join or hybridise
B.	Denaturation of DNA	2.	Polymerisation
C.	Annealing	3.	*Thermus aquaticus*
D.	Extension	4.	Kary Mullis

Codes

	A	B	C	D		A	B	C	D
(a)	4	3	2	1	(b)	1	2	3	4
(c)	3	1	2	4	(d)	4	3	1	2

140 Match the following columns.

	Column I		Column II
A.	Plasmids	1.	Natural polymer from sea water
B.	Bacteriophages	2.	Hybrid vector derived from plasmids
C.	Cosmids	3.	Virus infecting bacteria
D.	Agarose	4.	Circular, extrachromosomal DNA

Codes

	A	B	C	D		A	B	C	D
(a)	2	1	3	4	(b)	4	3	2	1
(c)	3	2	1	4	(d)	1	4	3	2

141 Match the following columns.

	Column I (Scientists)		Column II (Contributions)
A.	Arber, Nathan and Hamilton Smith	1.	Term biotechnology
B.	Paul Berg	2.	First recombinant DNA
C.	Herbert Boyer and Stanley Cohen	3.	Father of genetic engineering
D.	Karl Erkey	4.	Isolated first restriction endonuclease from bacteria

Codes

	A	B	C	D		A	B	C	D
(a)	1	4	3	2	(b)	3	2	1	4
(c)	4	3	2	1	(d)	4	3	1	2

142 Match the following columns.

	Column I (Vectors)		Column II (Derivative microorganisms)
A.	*Eco* RI	1.	*E. coli* R 245
B.	*Hind* III	2.	*Bacillus amyloliquefaciens*
C.	*Bam* HI	3.	*Haemophilus influenzae*
D.	*Eco* RII	4.	*Escherichia coli* RY13

Codes

	A	B	C	D
(a)	1	2	3	4
(b)	3	2	1	4
(c)	4	3	2	1
(d)	4	2	3	1

143 Match the following enzymes with their functions. ***NEET (Odisha) 2019***

	Column I		Column II
A.	Restriction endonuclease	1.	Joins the DNA fragments
B.	Restriction exonuclease	2.	Extends primers on genomic DNA template
C.	DNA ligase	3.	Cuts DNA at specific position
D.	*Taq* polymerase	4.	Removes nucleotides from the ends of DNA

Codes

	A	B	C	D		A	B	C	D
(a)	3	1	4	2	(b)	3	4	1	2
(c)	4	3	1	2	(d)	2	4	1	3

144 Match the following columns and choose the correct option from the codes given below. ***AIIMS 2019***

	Column I (Substrates)		Column II (Enzymes)
A.	Ribonucleotide	1.	Chitinase
B.	Chitin	2.	Cellulase
C.	Cellulose	3.	Ribonuclease

Codes

	A	B	C
(a)	1	2	3
(b)	3	1	2
(c)	3	2	1
(d)	2	1	3

NCERT Exemplar

MULTIPLE CHOICE QUESTIONS

145 A bacterial cell was transformed with a recombinant DNA molecule that was generated using a human gene. However, the transformed cells did not produce the desired protein. Reasons could be
(a) human gene may have intron which bacteria cannot process
(b) amino acid codons for humans and bacteria are different
(c) human protein is formed but degraded by bacteria
(d) All of the above

146 'Restriction' in restriction enzyme refers to
(a) cleaving of phosphodiester bond in DNA by the enzyme
(b) cutting of DNA at specific position only
(c) prevention of the multiplication of bacteriophage by the host bacteria
(d) All of the above

147 Which of the following bacteria is not a source of restriction endonuclease?
(a) *Haemophilus influenzae*
(b) *Escherichia coli*
(c) *Agrobacterium tumefaciens*
(d) *Bacillus amyloliquefaciens*

148 Which of the following enzymes catalyses the removal of nucleotides from the ends of DNA?
(a) endonuclease (b) exonuclease
(c) DNA ligase (d) *Hin*d II

149 Which of the following statements does not hold true for restriction enzyme?
(a) It recognises a palindromic nucleotide sequence
(b) It is an endonuclease
(c) It is isolated from viruses
(d) It can produce the same kind of sticky ends in different DNA molecules

150 Which of the following is not required in the preparation of a recombinant DNA molecule?
(a) Restriction endonuclease (b) DNA ligase
(c) DNA fragments (d) *E. coli*

151 The role of DNA ligase in the construction of a recombinant DNA molecule is
(a) formation of phosphodiester bond between two DNA fragments
(b) formation of hydrogen bonds between sticky ends of DNA fragments
(c) ligation of all purine and pyrimidine bases
(d) None of the above

152 In agarose gel electrophoresis, DNA molecules are separated on the basis of their
(a) charge only (b) size only
(c) charge to size ratio (d) All of these

153 Which of the given statement is correct in the context of visualising DNA molecules separated by agarose gel electrophoresis?
(a) DNA can be seen in visible light
(b) DNA can be seen without staining in visible light
(c) Ethidium bromide stained DNA can be seen in visible light
(d) Ethidium bromide stained DNA can be seen under exposure to UV-light

154 The most important feature in a plasmid to serve as a vector in gene cloning experiment is
(a) origin of replication (*ori*)
(b) presence of a selectable marker
(c) presence of sites for restriction endonuclease
(d) its size

155 An antibiotic resistance gene in a vector usually helps in the selection of
(a) competent bacterial cells
(b) transformed bacterial cells
(c) recombinant bacterial cells
(d) None of the above

156 The transfer of genetic material from one bacterium to another through the mediation of a viral vector is termed as
(a) transduction
(b) conjugation
(c) transformation
(d) translation

157 Significance of heat shock method in bacterial transformation is to facilitate
(a) binding of DNA to the cell wall
(b) uptake of DNA through membrane transport proteins
(c) uptake of DNA through transient pores in the bacterial cell wall
(d) expression of antibiotic resistance gene

158 While isolating DNA from bacteria, which of the following enzymes is not required?
(a) Lysozyme
(b) Ribonuclease
(c) Deoxyribonuclease
(d) Protease

159 Which of the following contributed popularising the PCR (Polymerase Chain Reaction) technique?
(a) Easy availability of DNA template
(b) Availability of synthetic primers
(c) Availability of cheap deoxyribonucleotides
(d) Availability of 'thermostable' DNA polymerase

160 Who among the following was awarded the Nobel Prize for the development of PCR technique?
(a) Herbert Boyer (b) Har Govind Khorana
(c) Kary Mullis (d) Arthur Kornberg

161 Which of the following steps are catalysed by *Taq* polymerase in a PCR reaction?
(a) Denaturation of template DNA
(b) Annealing of primers to template DNA
(c) Extension of primer end on the template DNA
(d) All of the above

162 Which of the following should be chosen for best yield if one were to produce a recombinant protein in large amounts?
(a) Laboratory flask of largest capacity
(b) A stirred-tank bioreactor without in-lets and out-lets
(c) A continuous culture system
(d) All of the above

Answers

› Mastering NCERT with MCQs

1 (c)	2 (d)	3 (a)	4 (d)	5 (a)	6 (a)	7 (b)	8 (c)	9 (b)	10 (d)
11 (a)	12 (b)	13 (d)	14 (c)	15 (b)	16 (d)	17 (c)	18 (c)	19 (a)	20 (a)
21 (c)	22 (a)	23 (c)	24 (c)	25 (c)	26 (b)	27 (b)	28 (a)	29 (b)	30 (c)
31 (b)	32 (b)	33 (b)	34 (b)	35 (d)	36 (c)	37 (a)	38 (d)	39 (b)	40 (a)
41 (c)	42 (d)	43 (a)	44 (b)	45 (b)	46 (a)	47 (d)	48 (b)	49 (b)	50 (a)
51 (b)	52 (c)	53 (b)	54 (c)	55 (c)	56 (d)	57 (a)	58 (a)	59 (d)	60 (a)
61 (d)	62 (a)	63 (a)	64 (c)	65 (a)	66 (b)	67 (b)	68 (b)	69 (a)	70 (b)
71 (b)	72 (b)	73 (c)	74 (b)	75 (b)	76 (b)	77 (d)	78 (a)	79 (d)	80 (a)
81 (b)	82 (b)	83 (a)	84 (d)	85 (a)	86 (a)	87 (b)	88 (d)	89 (a)	90 (d)
91 (d)	92 (d)	93 (a)	94 (c)	95 (c)	96 (d)	97 (d)	98 (c)	99 (b)	100 (a)
101 (c)	102 (d)	103 (b)	104 (d)						

› NEET Special Types Questions

105 (c)	106 (a)	107 (a)	108 (d)	109 (a)	110 (b)	111 (c)	112 (c)	113 (a)	114 (d)
115 (a)	116 (c)	117 (b)	118 (d)	119 (c)	120 (c)	121 (d)	122 (c)	123 (d)	124 (d)
125 (a)	126 (d)	127 (a)	128 (b)	129 (c)	130 (d)	131 (d)	132 (d)	133 (c)	134 (c)
135 (a)	136 (b)	137 (c)	138 (b)	139 (d)	140 (b)	141 (c)	142 (c)	143 (b)	144 (b)

› NCERT Exemplar Questions

145 (a)	146 (b)	147 (c)	148 (b)	149 (c)	150 (d)	151 (a)	152 (b)	153 (d)	154 (a)
155 (b)	156 (a)	157 (c)	158 (c)	159 (d)	160 (c)	161 (c)	162 (c)		

Answers & Explanations

5 (*a*) The two core technologies that enabled birth of modern biotechnology are

- Bioprocess engineering, i.e. processes that help the growth of the desired prokaryotic or eukaryotic cell in large quantities in a sterile medium for the manufacture and multiplication of biotechnological products.
- Genetic engineering, which is the process of manipulation of genes, to introduce into the host organisms.

6 (*a*) Genetic engineering includes techniques which alter the chemistry of genetic material (DNA and RNA) to induce the expression of genes which will give the desired products.

10 (*d*) Stanley Cohen and Herbert Boyer generated the first recombinant DNA molecule by linking a gene encoding antibiotic resistance with a native plasmid of *Salmonella typhimurium*.

11 (*a*) The linking of the anitbiotic resistance genes with the plasmid vector became possible with DNA ligase. It is the enzyme that joins two complementary nucleotides.

12 (*b*) The correct sequence of step of genetic engineering are as follows

- The first step involves the identification of DNA with desirable genes.
- These genes are introduced into the host and are maintained in the host.
- Finally, the transfer of this DNA to the host progeny takes place.

14 (*c*) The enzymes, commonly used in genetic engineering are restriction endonuclease and ligase. Restriction endonuclease make cuts at specific positions within the DNA and these DNA fragments can be joined together end-to-end by using ligase enzyme.

16 (*d*) *Hin*d II is a restriction endonuclease. It was the first restriction endonuclease to be discovered. Restriction endonucleases are the enzymes which are used for cutting of DNA at specific location.

17 (*c*) The restriction enzyme responsible for the cleavage of the given sequence is *Hin*d II.

$$\begin{matrix} & \downarrow & \\ 5' - \text{GTC} & & \text{GAC} - 3' \\ 3' - \text{CAG} & & \text{CTG} - 5' \\ & \uparrow & \end{matrix}$$

20 (*a*) The convention for naming restriction enzymes is as follows

The first letter of the name comes from the genus (A), the second two letters come from the species (B) and the fourth letter comes from the strain (C) of bacteria (D), e.g. *Eco* RI comes from *Escherichia coli* RY13.

23 (*c*) Restriction enzymes belong to a larger class of enzymes called nucleases, which are of two kinds, i.e. exonucleases and endonucleases.

25 (*c*) An enzyme catalysing the removal of nucleotides from ends of DNA is an exonuclease.

Other options are

- Endonucleases make cuts at specific positions within the DNA. DNA ligase joins the DNA fragments.
- Proteases are protein-degrading enzymes.
- DNA ligase joins two complementary single-stranded DNA molecules.

26 (*b*) Restriction endonucleases recognise a specific DNA base sequence (recognition sequence, recognition site), bind to the DNA and then cleave both the strands of the DNA at those specific points.

27 (*b*) Restriction endonuclease enzymes cut double-stranded DNA molecules at specific sites called recognition sites which have specific base sequences.

28 (*a*) Restriction endonucleases recognise their specific sequence and bind to the DNA and cut each of the two strands of the double helix at specific points in their sugar-phosphate backbone.

30 (*c*) Palindrome in DNA is the sequence of base pairs which read same on the two strands when orientation of reading is kept the same, e.g.

$$\begin{matrix} 5' - \text{GAATTC} - 3' \\ 3' - \text{CTTAAG} - 5' \end{matrix}$$

The sequence given above reads the same on both the strands either in $5' \rightarrow 3'$ direction or in $3' \rightarrow 5'$ direction.

31 (*b*) Restriction enzymes cut DNA strands a little away from the centre of the palindrome site between the same two bases on the opposite strands.

32 (*b*) Same restriction enzymes are used to cut both the foreign DNA and the vector, so that the resultant DNA fragments have the complementary kind of sticky ends.

33 (*b*) If a linear DNA molecule is digested using a restriction enzyme having 4 recognition sites, it will produce 5 fragments.

34 (*b*) When a closed circular DNA molecule is digested with a restriction enzyme having six recognition sites, it will produce 6 DNA fragments.

35 (*d*) DNA ligases (genetic gum) are used in recombinant DNA technology to join two individual fragments of double-stranded DNA by forming phosphodiester bonds between them to produce a recombinant DNA (plasmid).

36 (*c*) The enzyme DNA ligase is also called as molecular glue, as it works to repair broken DNA by joining two complementary nucleotides in a DNA strand. It is commonly used in genetic engineering to do the reverse of restriction enzyme, i.e. to join together complementary restriction fragments.

Other options are

- DNA gyrase is an enzyme of class topoisomerase that relieves torque or strain in DNA during DNA replication.
- DNA helicase unwinds the double helix to make site of DNA synthesis.
- DNA polymerase catalyses the formation of new DNA on template strand.

37 (*a*) Enzyme A is *Eco* RI which is used in step I and enzyme B is DNA ligase, used in step II. *Eco* RI cuts the DNA between bases G and A only when the sequence GAATTC is present in the DNA. This enzyme cuts both DNA strands at the same site. Enzyme ligase joins the complementary DNA fragments at their sticky ends to form the recombinant DNA.

42 (*d*) A molecule of DNA can be cut into fragments by the enzyme restriction endonucleases. These fragments of DNA can be separated on the basis of size by a technique of gel electrophoresis. It is the most common method used to separate DNA molecules on the basis of their size.

43 (*a*) Agarose is extracted from sea weeds and is a polysaccharide. In gel electrophoresis, DNA fragments separate according to their size through seiving effects provided by the agarose gel.

44 (*b*) Gel electrophoresis is used for the separation of molecules of similar electric charge on the basis of their size. Hence, smaller size of the DNA fragment, the farther it moves on agarose gel during gel electrophoresis.

45 (*b*) DNA being negatively charged migrates towards the positive terminal, the anode, but is loaded at cathode.

46 (*a*) The smallest fragments of DNA are found near the positive electrodes as DNA is negatively charged. These fragments travel towards anode (farthest away from the leading wells).

47 (*d*) The DNA fragments separated on an agarose gel can be visualised after staining with ethidium bromide. It is an intercalating, fluorescent agent. The stained DNA fragments are seen as bright orange coloured bands under UV light.

51 (*b*) In recombinant DNA technology, vector refers to a plasmid used to transfer foreign DNA into a host cell, where the genes may be amplified (gene cloning) or otherwise manipulated.

52 (*c*) Bacteriophage are used in recombinant DNA technique. These are used as vectors due to their high number per cell and high copy numbers of their genome within the bacterial cells.

53 (*b*) During 'gene cloning' the plasmid vector is called a gene taxi. Desired genes are inserted into plasmids, then the plasmid with the added gene are delivered into a living bacterium.

54 (*c*) Plasmid is not single-stranded. Plasmid is an extrachromosomal, double-stranded, circular DNA molecule, having the ability of self-replication. These are usually found in bacterial cells and from some yeasts. Discovery of plasmid has led to the revolution in the biotechnological research.

55 (*c*) Plasmid is a vector which can clone small DNA fragments. Small fragment of DNA (about 10 Kbp size) that is physically separate from and can replicate freely of chromosomal DNA within a cell.

57 (*a*) pBR322 is a plasmid vector. A plasmid is an extrachromosomal genetic elements of DNA that is capable of replicating independently of the host chromosome.

59 (*d*) Option (d) contains the correctly identified components as amp^R (ampicillin resistance gene) and tet^R (tetracycline resistance gene) are antibiotic resistance genes. Rest of the options are incorrect and can be corrected as

- *Ori* is origin of replication,
- *rop* codes for proteins involved in replication of the plasmid and stands for regulation of origin of plasmid.
- *Hin*d III as well as *Eco* RI are restriction sites.

60 (*a*) The two antibiotic resistance genes on the cloning vector pBR322 are for ampicillin and tetracycline antibiotics. Cloning vectors are DNA molecules that carry a foreign DNA segment and replicate inside host cell. Plasmid in *E.coli* is a cloning vector.

61 (*d*) Origin of replication is the sequence from where replication starts and any piece of DNA when linked to this sequence can be made to replicate within host cells.

This sequence is also responsible for controlling the copy number of the linked DNA. Thus, the function of *ori* in a vector is to help in replication of linked DNA and control the copy number of the linked DNA.

62 (*a*) A gene, whose expression helps to identify transformed cells is known as selectable marker. Usually, the genes encoding resistance to antibiotics, such as tetracycline, ampicillin, etc., are called selectable markers (for *E. coli*).

63 (*a*) To facilitate cloning, a vector requires a selectable marker, which helps in identifying and eliminating non-transformants and selectively permitting the growth of the transformants.

64 (*c*) Gene encoding resistance to antibiotics like ampicillin, chloramphenicol, tetracycline or kanamycin, useful for cloning are called selectable markers. These are suitable selectable markers for *E. coli* as the normal *E. coli* cells do not carry resistance against any of these antibiotics.

65 (*a*) The presence of more than one recognition sites within the vector will generate several fragments, which will complicate the gene cloning process.

68 (*b*) Insertional inactivation is used to differentiate recombinants and non- recombinants, on the basis of the inability of the recombinants to produce colour in the presence of a chromogenic substrate.

69 (*a*) In insertional inactivation, the recombinant DNA is inserted within the coding sequence of the enzyme β-galactosidase. This results into inactivation of the gene synthesising this enzyme.

70 (*b*) Recombinant colonies in insertional inactivation are differentiated from non-recombinants on the basis of their inability to produce the blue colour in the presence of a chromogenic substrate. The presence of a chromogenic substrate gives blue coloured colonies, if the plasmid in bacteria does not have an insert. The presence of insert results in the inactivation of enzyme β-galactosidase due to which colourless recombinant colonies are produced.

71 (*b*) *Agrobacterium tumefaciens,* a pathogen of several dicot plants is able to deliver a piece of DNA known as T-DNA to transform normal plant cells into tumour cells.

74 (*b*) In Ti plasmid, the virulent gene is removed to make it a suitable (competent) vector for gene transfer. In it, tumour forming virulent genes are replaced with desired genes and this way it acts as a vector to carry the desired gene to the host organism where it gets expressed to produce the desired product.

76 (*b*) Host is made competent by treating them with specific concentration of a divalent cation such as calcium, which increases the efficiency with which DNA enters the bacterium through the creation of pores in its cell wall or cell membrane.

79 (*d*) Biolistics or gene gun method is a direct or vector less way used to introduce alien DNA into host cells. In this method of gene transfer, high velocity micro particles of gold or tungsten, coated with DNA are bombarded on the desired host cells which are usually plant cells.

80 (*a*) DNA transfer with high velocity microparticles is present in biolistics. This technique involves bombardment of microscopic gold particles coated with the foreign DNA at the cells using a compressed air gun. It is designed to overcome the high impermeability of cell wall in plants. These particles can penetrate the cell wall, the cell and the nuclear membrane to deliver the DNA to the nucleus.

82 (*b*) In bacterial cells, the cell wall (membrane) is digested with the help of the enzyme lysozyme. On the other hand,

- Cellulase and chitinase enzymes are used for the digestion of plant and fungal cell walls, respectively.
- Lipids can be removed with the treatment of the enzyme lipase.

85 (*a*) Chilled ethanol is used to precipitate DNA out of a mixture of biomolecules. Low temperature protects the DNA by slowing down the activity of enzymes that could break it apart and ethanol helps in the quick precipitation of DNA.

87 (*b*) A chimeric DNA is a recombinant DNA generated by ligating sequences derived from different sources.

88 (*d*) PCR is a technique of synthesising multiple copies of the desired gene (or DNA) of interest *in vitro*. The basic requirements of PCR are DNA template, two sets of primers and the enzyme (*Taq* polymerase).

90 (*d*) *Taq* polymerase is a thermostable DNA polymerase obtained from *Thermus aquaticus*. This enzyme is not denatured at high temperature. *Thermus aquaticus* is a bacterium that lives in hot springs and hydrothermal vents at a temperature of 300ºC due to the presence of this enzyme.

94 (*c*) Transformation is a procedure through which a piece of DNA is introduced in a host bacterium. Cells containing DNA with ampicillin resistance gene (transformed recipient cells) when introduced into agar plates containing ampicillin will grow, while cells containing DNA without ampicillin resistance gene (untransformed recipient cells) will die. The genes encoding resistance to antibiotics such as ampicillin, chloramphenicol, tetracycline or kanamycin, etc., are hence, considered as useful selectable markers for *E.coli*. The normal *E. coli* cells do not carry resistance against any of these antibiotics.

96 (*d*) In continuous culture system, the used medium is continuously drained out from one side, while simultaneously fresh medium is added from the other to maintain the cells in their physiologically most active log phase. This type of method produces a larger biomass leading to higher yield of desired products.

99 (*b*) A stirred-tank bioreactor is more advantageous than shake flasks as these provide better aeration and mixing properties. It has an agitator system to mix the contents properly and also an oxygen delivery and also system to ensure better availability of oxygen.

103 (*b*) The process of separation and purification of expressed proteins before marketing is called as downstream processing. In this process, a whole range of biochemical separation and purification techniques are used such as drying, chromatography, solvent extraction and distillation. After purification, several quality control testings are done so as to make the product suitable for marketing.

104 (*d*) Expression is not a component of downstream processing. Infact, processing, separation and purification are collectively referred to as downstream processing and the product has to be formulated with suitable preservatives. The product has to be subjected through this complete series before it is ready for marketing as a finished product.

105 (*c*) Assertion is true, but Reason is false. Reason can be corrected as

Biotechnology uses unicellular as well as multicellular organisms.

Biotechnology deals with techniques that use living organisms to produce products useful for humans.

106 (*a*) Both Assertion and Reason are true and Reason is the correct explanation of Assertion.

Maintenance of sterile (microbial contamination-free) environment is essential in biotechnological processes to enable growth of only the desired prokaryotic or eukaryotic cells in large quantities for manufacture of biotechnological products like antibiotics, vaccines, enzymes, etc.

107 (*a*) Both Assertion and Reason are true and Reason is the correct explanation of Assertion.

Origin of replication (*ori*) is the sequence from where replication starts and any piece of DNA when linked to this sequence can be made to replicate within the host cell. Thus, it is an essential part of a vector.

108 (*d*) Assertion is false, but Reason is true. Assertion can be corrected as

In the formation of *r*DNA, restriction endonucleases cut both foreign DNA and vector DNA.

Whereas, ligases join two DNA strands by forming phosphodiester bonds between adjacent nucleotides.

109 (*a*) Both Assertion and Reason are true and Reason is the correct explanation of Assertion.

DNA fragments can be isolated with the help of gel electrophoresis. In this technique, negatively charged DNA moves towards the anode (positively charged) under an electric field through a matrix.

110 (*b*) Both Assertion and Reason are true, but Reason is not the correct explanation of Assertion.

Restriction endonucleases are molecular scissors, as these enzymes cut a DNA molecule within certain specific sites called restriction sites.

When these fragments with sticky ends are mixed, they can be joined together (end to-end) using the enzyme, DNA ligase.

111 (*c*) Assertion is true, but Reason is false. Reason can be corrected as

Restriction endonucleases were discovered from bacteria, not from viruses.

All endonucleases cut DNA at specific sites within the DNA sequence.

112 (*c*) Assertion is true, but Reason is false. Reason can be corrected as

The tumour inducing, Ti plasmid of *Agrobacterium tumefaciens* has been modified into a cloning vector which is not pathogenic to the plants. However, it can still used to deliver genes of interest into target plant cells.

113 (*a*) Both Assertion and Reason are true and Reason is the correct explanation of Assertion.

Fungal cell wall is made up of chitin. Thus, enzyme chitinase is required for the digestion of fungal cell wall and isolation of DNA from the cell.

114 (*d*) The statement in option (d) is correct about DNA polymerase used in PCR. Rest of the statements are incorrect and can be corrected as

- It is used to catalyse DNA synthesis.
- It does not serve as a selectable marker.
- It is isolated from a bacterium, *Thermus aquaticus*.

115 (*a*) The statement in option (a) is incorrect. It can be corrected as

Restriction endonuclease enzymes cut both the strands of DNA helix at specific sites in their sugar-phosphate backbone. The sequences being recognised by restriction enzymes are called palindromic sequences which have same reading frame in both $5' \rightarrow 3'$ and $3' \rightarrow 5'$ directions.

Rest of the statements are correct.

116 (*c*) The statements in option (c) is incorrect and can be corrected as

Genetic engineering helps in making recombinant DNA and not artificial limbs and diagnostic instruments.

Rest of the statements are correct.

117 (*b*) The statement in option (b) is incorrect and can be corrected as

Agrobacterium tumefaciens delivers a piece of DNA known as 'T-DNA', which transforms normal plant cells into tumour cells and directs these tumour cells to produce the chemicals required by the pathogen.

Rest of the statements are true.

119 (*c*) The statement in option (c) is incorrect and can be corrected as

Restriction enzymes belong to a larger class of enzymes called nuclease and can cut DNA a little away from the center of the palindromic sites, but between the same two bases on the opposite strands.

Rest of the statements are correct.

120 (*c*) The statement in option (c) is incorrect and can be corrected as

pBR322 was the first artificial ideal plasmid (vector) constructed by Boliver and Rodriguez.
Rest of the statements are correct.

121 (*d*) The statement in option (d) is incorrect and can be corrected as

Some plasmids may have only one or two copies per cell whereas others may have 15-100 copies per cell.

Rest of the statements are correct.

122 (*c*) Statements in option (c) is incorrect and can be corrected as

Electroporation involves an electric pulse of high voltage applied to protoplasts/cells/tissues to make transient (temporary) pores in the plasma membrane which facilitates the uptake of foreign DNA.

Rest of the statements are correct.

124 (*d*) Statements I, II and IV are incorrect and can be corrected as

- DNA fragments are negatively charged molecules and are loaded in the middle of the gel. The fragments can be separated by forcing them to move towards the anode under an electric field through a medium/matrix.
- The concentration of gel affects the resolution of DNA separation.

- The separated DNA fragments can be visualised only after staining the DNA with a compound known as ethidium bromide followed by exposure to UV-radiation.

125 (*a*) Statements I, II and III are correct, while statement IV is incorrect and can be corrected as

Largest DNA bands will be at (A) and the smallest DNA bands will be at (B) because in this technique, DNA moves according to their size through sieving effect provided by the agarose gel. Hence, the smaller the fragment size, the farther it moves.

128 (*b*) Statement I is false, but II is true. Statement I can be corrected as

In the process of recombinant DNA technology, after several treatment the purified DNA is precipitated by adding chilled ethanol.

129 (*c*) Statements II and III are correct. Statement I is incorrect and can be corrected as

Large volumes (100-1000 L) of culture can be processed in bioreactors. A bioreactor provides the optimal conditions for obtaining the desired product. In the sparged stirred-tank bioreactor, sterile air bubbles are sparged. This increases the surface area for oxygen transfer.

145 (*a*) Inducing a cloned eukaryotic (human) gene to function in a prokaryotic (bacteria) host can be difficult sometimes.

The presence of long non-coding introns in eukaryotic genes may prevent the correct expression of these genes in prokaryotes as they lack the RNA-splicing machinery.

147 (*c*) *Agrobacterium tumefaciens* is not a source of restriction endonuclease but serves as the source of Ti plasmid.

On the other hand, *Hin*d III is obtained from *Haemophilius influenzae*, *Eco* RI obtained from *Escherichia coli* and *Bam* HI obtained from *Bacillus amyloliquefaciens*.

149 (*c*) The statement in option (c) is not true for restriction enzymes. It can be corrected as

It is isolated from bacteria and not from viruses.

Rest other statements are correct.

150 (*d*) *E. coli* is not required in the preparation of recombinant DNA molecules.

On the other hand, restriction endonuclease and DNA ligase can be used to make a stable recombinant DNA molecule with DNA fragments that have been obtained from different organisms.

153 (*d*) The separated DNA fragments (by the process of gel electrophoresis) are visualised after staining the DNA fragments with ethidium bromide followed by exposure to UV-radiation. These fragments are seen as orange coloured bands (light).

154 (*a*) All of the given features of a vector are important to facilitate cloning, but out of them origin of replication (*ori*) is the most important one.

This is due to the following reasons

- *ori* is a DNA sequence that is responsible for initiating replication. Any piece of DNA when linked to this sequence can replicate within the host cells.
- *ori* also controls the copy numbers of the linked DNA.

155 (*b*) An antibiotic resistance gene in a vector acts as a selectable marker and usually helps in the selection of transformed bacterial cells.

Selectable markers in a vector help in identifying and eliminating non-transformants and selectively permitting the growth of the transformants.

156 (*a*) Transduction is the process by which genetic material is transferred from one bacterium to another through the mediation of viral vectors.

157 (*c*) In heat shock method, firstly the cells with recombinant DNA are placed on ice, followed by exposing them briefly to a temperature of 42°C (heat shock) and then putting them back on ice. This enables uptake of DNA through transient pores created in the bacterial cell wall.

159 (*d*) Polymerase Chain Reaction (PCR) involves amplification of specific DNA sequences, carried out *in vitro*.

Such repeated amplification is achieved by the use of a thermostable DNA polymerase (isolated from a bacterium (*Thermus aquaticus*), which remains active and stable during the high temperature and induced denaturation of double-stranded DNA. Thus, availability of thermostable DNA polymerase has popularised PCR.

160 (*c*) PCR (Polymerase Chain Reaction) technique was developed by Kary Mullis in 1985 and for this he received Nobel Prize for chemistry in 1993.

Other options are

- HG Khorana discovered DNA ligase enzyme into phage in 1969.
- DNA polymerase was discovered by Arthur Kornberg.
- Herbert Boyer generated the first recombinant DNA molecule by combining a gene from a bacterium with plasmid of *E. coli* in 1972.