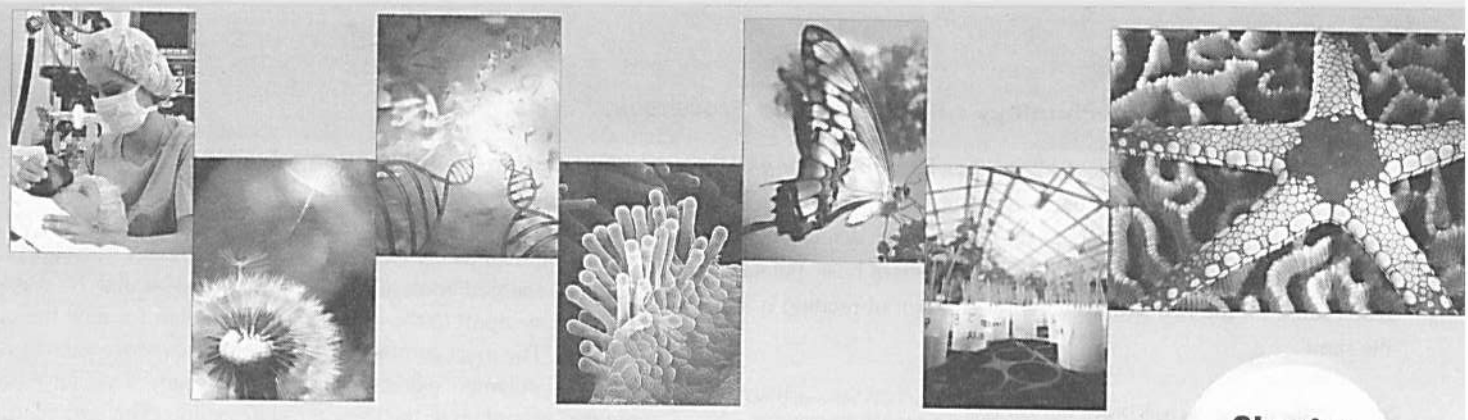


Chapter

9.1

Biotechnology : Principles and Processes

Recombinant DNA Technology

It is a technology that allows DNA to be produced via artificial means.

It is the joining together of DNA molecules from two different species that are inserted into a host organism to produce new genetic combinations that are of value to science, medicine agriculture and industry.

This technology works by taking DNA from two different sources and combining it into a single molecule. That alone, however will not do much. It only becomes useful when that artificially created DNA is 111 reproduced in a process known as DNA cloning.

There are two main types of cloning that recombinant DNA technology is used for therapeutic cloning & reproductive cloning most people are familiar with reproductive cloning. Which will produce an organism with the exact genetic information of one that already exist. This has already been done with some animals. Dolly a Sheep, was the first mammal to ever be reproduced as an exact genetic copy.

Recombinant DNA technology is not accepted by some people, especially social conservatives who feel the technology is a slippery slope to devaluing the uniqueness of life. Further, because some DNA work involves the use and destruction of embryos. It attracts even more controversy still, proponents of the technology say the ultimate goal is to benefit human life, not destroy it.

Recombinant DNA technology is widely used in biotechnology medicine and research. Today recombinant proteins and other products that result from the use of rDNA technology are found in essentially every western pharmacy, doctor's, medical testing laboratory and biological research laboratory. In addition, organisms that have been manipulated using rDNA technology and products derived from those organisms have found their way into many farms supermarkets, home, medicine cabinets and even pet shops.

Genetic engineering

Recombinant DNA technology : Genetic engineering, a kind of biotechnology, is the latest branch in applied genetics dealing the alteration of the genetic make up of cells by deliberate and artificial means. Genetic engineering involves transfer or replacement of genes, so also known as recombination DNA technology or gene splicing.

Tools of Recombinant DNA Technology : Now we know from the foregoing discussion that 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. Let us try to understand some of these in detail.

Restriction Endonucleases : In 1963, two enzymes responsible for restricting the growth of bacteriophage in *Escherichia coli* were isolated. One of these added methyl groups to DNA, while the other cut DNA. The later was called **restriction endonucleases.**

The First Restriction Endonucleases : *Hind II*, whose functioning depended on a specific DNA nucleotide sequence was isolated and characterized five years later. It was found that *Hind II* always cut DNA molecules at a particular point by recognizing a specific sequence of six base pairs. This specific base sequence is known as the recognition sequence for *Hind II*. Besides *Hind II* today we know more than 900 restriction enzymes that have been isolated from over 230 strains of bacteria each of which recognize different recognition sequences. The convention of naming these enzymes is the first letter of the name comes from the genus and the second two letters come from the species of the prokaryotic cell from which they were isolated, *e.g.*, EcoRI comes from *Escherichia coli* RY 13. In EcoRI, the letter 'R' is derived from the name of strain. Roman numbers following the names indicate the order in which the enzymes were isolated from the strain of bacteria.

Palindromes are groups of letters that form the same words when read both forward and backward, *e.g.*, "MALAYALAM". As against a word-palindrome where the same word is read in both directions, the palindrome in DNA is a sequence of basic pairs that reads same on the two strands when orientation of reading is kept the same.

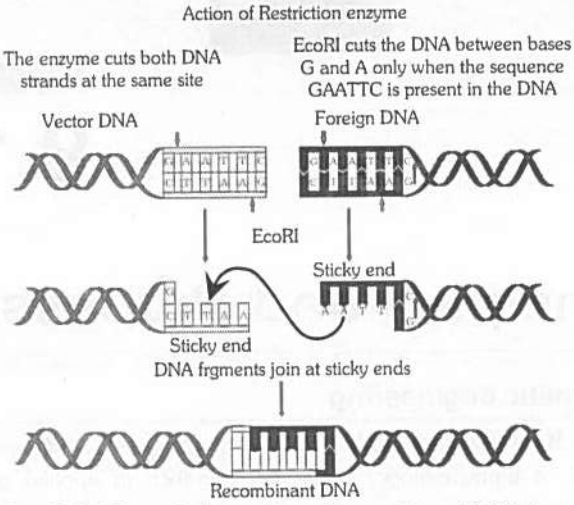


Fig : 9.1-1 Steps in formation of recombinant DNA by action of restriction endonuclesase enzyme - EcoRI

For example, the following sequence reads the same on the two strands in 5'→3' direction. This is also true if read in the 3'→5' direction.

5'——— GAATTC ———3'
3'——— CTTAAG ———5'

Restriction enzymes cut the strand of DNA a little away from the centre of the palindrome sites, but between the same two bases on the opposite strands. This leaves single stranded positions at the ends. There are overhanging stretches called **sticky ends** on each strand. These are named so because they form hydrogen bonds with their complementary cut counterparts. This stickiness of the ends facilitates the action of the enzyme DNA ligase. Such enzymes help in creating recombinant molecules of DNA.

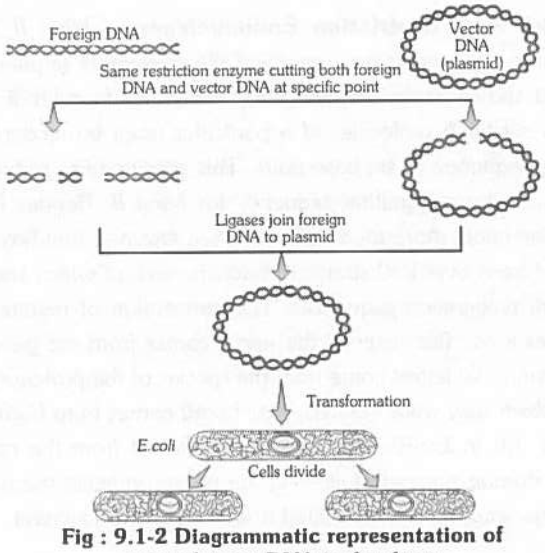


Fig : 9.1-2 Diagrammatic representation of recombinant DNA technology

Separation and Isolation of DNA Fragments : The cutting of DNA by restriction endonucleases results in the fragments of DNA. These fragments can be separated by a technique known as gel electrophoresis. Since DNA fragments are negatively charged molecules they can be separated by forcing them to move apart to the anode through the electric field through a medium. The most commonly used matrix is agarose gel which is a natural polymer extracted from sea weed. The DNA are separated according to their size through sieving effect provided by the agarose gel. The smaller the fragment the farther it moves. The separated DNA can be visualized only after staining the DNA with a compound known as ethidium bromide followed by exposer to UV radiations Bright orange coloured bands of DNA in a ethidium bromide stained gel exposed to UV light. The separated bands of DNA are cut out from the agarose gel and extracted from the gel piece. This is called **elution**. The DNA fragment is purified and used in constructing DNA recombinant.

Cloning Vectors : Once an alien DNA has been added to the host DNA, then the right vector is needed which can pass this DNA to a host cell for multiplication of the alien DNA. The choice can be of a bacteriophage or a plasmid. A bacteriophage makes many copies per cell. Some plasmids may have only one or two copies per cell whereas others may have 15 – 100 copies per cell. Vectors used at present are engineered in such a way that they help easy linking of foreign DNA and selection of recombinants from non-recombinants. The following are the features required to facilitate cloning into a vector.

(i) **Origin of replication (ori) :** This is a sequence from where replication starts and any piece of DNA when linked to this sequence can be made to replicate within the host cells. This sequence is also responsible for controlling the copy number of the linked DNA. So, if one wants to recover many copies of the target DNA it should be cloned in a vector whose origin support high copy number.

(ii) **Selectable marker :** In addition to 'ori', the vector requires a selectable marker, which helps in identifying and eliminating non-transformants and selectively permitting the growth of the transformants. **Transformation** is a procedure through which a piece of DNA is introduced in a host bacterium (you will study the process in subsequent section). Normally, the genes encoding resistance to antibiotics such as ampicillin, chloramphenicol, tetracycline or kanamycin etc., are considered useful selectable markers for *E. coli*. The normal *E. coli* cells do not carry resistance against any of these antibiotics.

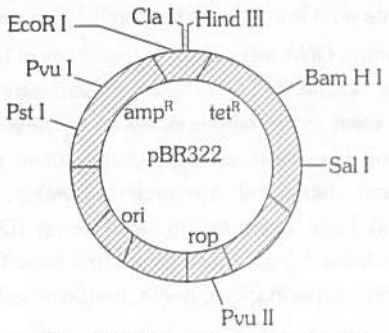


Fig : 9.1-3 E. coli cloning vector pBR322 showing restriction sites

(iii) **Cloning Sites** : In order to link the alien DNA, the vector needs to have very few, preferably single, **recognition sites** for the commonly used restriction enzymes. Presence of more than one recognition sites within the vector will generate several fragments, which will complicate the gene cloning. The ligation of alien DNA is carried out at a restriction site present in one of the two **antibiotic resistance** genes. For example, you can ligate a foreign DNA at the BamH I site of tetracycline resistance gene in the vector pBR322. The recombinant plasmids will lose tetracycline resistance due to insertion of foreign DNA but can still be selected out from non-recombinant ones by plating the transformants on ampicillin containing medium. The transformants growing on ampicillin containing medium are then transferred on a medium containing tetracycline. 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. In this case, one antibiotic resistance gene helps in selecting the transformants, whereas the other antibiotic resistance gene gets 'inactivated due to insertion' of alien DNA, and helps in selection of recombinants.

Selection of recombinants due to inactivation of antibiotics is a cumbersome procedure because it requires simultaneous plating on two plates having different antibiotics. Therefore, alternative selectable markers have been developed which differentiate recombinants from non-recombinants on the basis of their ability to produce colour in the presence of a chromogenic substrate. In this, a recombinant DNA is inserted within the coding sequence of an enzyme, β – galactosidase. This results into inactivation' of the enzyme, which is 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. Presence of insert results into insertional inactivation of the β – galactosidase and the colonies do not produce any colour, these are identified as recombinant colonies.

(iv) **Vectors for cloning genes in plants and animals :**

(a) Bacteria *Agrobacterium tumefaciens*, a pathogen of several dicot plants is able to deliver a piece of DNA known as T-DNA to transfer normal plant cells into a tumour and direct these tumour cells to produce the chemicals required by the pathogen.

(b) Retroviruses in animals have the ability to transform normal cells into cancerous cells. The tumour inducing (Ti) plasmid *Agrobacterium tumefaciens* has now been modified into a cloning vector which is no more pathogenic to the plants but is still able to use the mechanisms deliver genes of our interest into a variety of plants. Similarly, retroviruses have also been disarmed and are now used to deliver desirable genes into amnimal cells. Example – as in treating SCID patients.

Competent host (for transformation with recombinant DNA technology): Once DNA is introduced in the vector cell it needs a host cell so that it can be cloned. A bacterial cell is made competent enough to take up the DNA. This is done in following ways :

(i) By treating the bacterial cell with a specific concentration of a divalent cation, such as calcium, which increases the efficiency with which DNA enters the bacterium through pores in its cell wall. 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 degrees centigrade (heat shock) and then putting them back on ice. This enables the bacteria to take up the recombinant DNA.

(ii) **Micro-injection method** : Recombinant DNA is directly injected into the nucleus of an animal cell.

(iii) **Gene gun method** : In plant cells, bombardment on plant cell with high velocity micro particles of gold or tungsten coated with DNA in a method known as biolistic or gene gun.

(iv) Disarmed pathogen vectors are allowed to infect the cells, and transfer the recombinant DNA into the host.

Process of Recombinant DNA technology : It involves isolation of DNA, fragmentation of DNA by restriction endonucleases, isolation of desired DNA fragment, ligation of the DNA fragment into a vector, transferring the recombinant DNA into the host, culturing the host cells in a medium at large scale and extraction of the desired product.

(i) **Isolation of the genetic material (DNA)** : First the cells are broken up to release the DNA along with other macromolecules such as RNA, proteins, polysaccharides and lipids. It is achieved by treating the bacterial cells/ plant cells/ animal cells with enzymes like lysozymes (bacteria), cellulose (plant cells), and chitinase (fungus). RNA can be removed by treatment with ribonucleases whereas proteins can be removed by treatment with protease. Purified DNA precipitates out after the addition of chilled ethanol. It is seen as collection of fine threads in the suspension.

(ii) **Cutting of DNA at specific location** : Restriction enzyme digestions are performed by incubating purified DNA molecules with restriction enzymes, at the optimal conditions for that specific enzyme. Agarose gel electrophoresis is employed to check the progression of a restriction endonuclease digestion. DNA is a negatively charged molecule, hence it moves towards the positive electrode. This is repeated with the vector DNA also. The cut out gene of interest from the source DNA and the cut vector with space are mixed and ligase is added. Thus recombinant DNA is prepared.

(iii) **Amplification of gene of interest using PCR** : PCR means polymerized chain reaction. Multiple copies of gene (or DNA) of interest is synthesized in vitro using two sets of primer (small chemically synthesized oligonucleotides that are complementary to the regions of DNA) and the enzyme DNA polymerase.

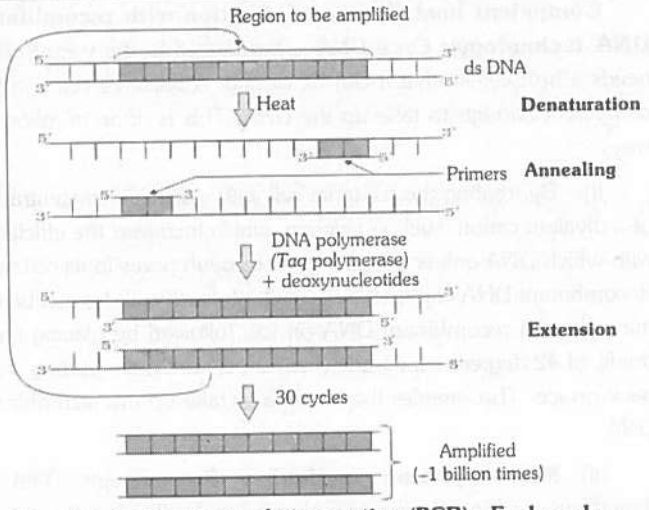


Fig : 9.1-4 Polymerase chain reaction (PCR) : Each cycle has three steps (i) Denaturation (ii) Primer annealing and (iii) Extension of primers

The enzyme extends the primers using the nucleotides provided in the reaction and the genomic DNA as the template. If the process of replication is repeated many times, the segment of DNA can be amplified to many times (billion times). Such repeats are possible by use of a thermostable DNA polymerase, isolated from a bacterium, *Thermus aquaticus*, which remains active during the high temperature induced denaturation of double stranded DNA. The amplified fragment if desired can be used to ligate with a vector for further cloning.

(iv) **Insertion of recombinant DNA into the Host cell/organism :** Insertion of recombinant DNA into host can be done in many ways *i.e.* by use of a vector, gene gun, microinjection etc. a selectable marker gene makes a cell competent enough to receive the recombinant DNA. Selectable marker can be an ampicillin resistance gene.

(v) **Obtaining the foreign gene product :** Cloning is done to obtain copies of the desired gene and its protein. It can be done on large scale. If any protein encoding gene is expressed in a heterologous host, it is called a recombinant protein. The cells harbouring cloned genes of interest may be grown on a small scale in the lab. The cultures may be used for extracting the desired protein and then purifying it by using different separation techniques. There are two ways to multiply the cells : (a) In a continuous culture – wherein the used medium is drained out from one side and fresh medium is added from the other side to maintain the cells in their physiologically most active/ log phase. This leads to higher biomass production or more protein synthesis. (b) To produce small quantity, simple fermenting vessel can be used.

Natural genetic engineer : When as gene transfer occurs without human effort, the bacterium is known as "natural genetic engineer" of plants. *e.g.*, A soil inhabiting, plant pathogenic bacterium, *Agrobacterium tumefaciens*.

Application of recombinant DNA technology : The technique of recombinant DNA can be employed in the following ways :

(1) It can be used to elucidate molecular events in the biological process such as cellular differentiation and ageing. The same can be used for making gene maps with precision.

(2) In biochemical and pharmaceutical industry, by engineering genes, useful chemical compounds can be produced cheaply and efficiently which is shown in table.

(3) Production of transgenic plants.

(4) Production of genetically modified microorganisms.

Table : 9.1-1 Applications of recombinant DNA products

Medically useful recombinant products	Applications
Human insulin	Treatment of insulin-dependent diabetes
Human growth hormone	Replacement of missing hormone in short stature people
Calcitonin	Treatment of rickets
Chronic gonadotropin	Treatment of infertility
Blood clotting factor VIII/IX	Replacement of clotting factor missing in patients with Haemophilia A/B
Tissue plasminogen activator	Dissolving blood clots after heart attacks and strokes
Erythropoitin	Stimulation of the formation of erythrocytes (RBCs) for patients suffering from anaemia during kidney dialysis or side effects of AIDS patients treated by drugs
Platelet derived growth factor	Stimulation of wound healing
Interferon	Treatment of pathogenic viral infections, cancer
Interleukins	Enhancement of action of immune system
Vaccines	Prevention of infectious diseases such as hepatitis B, herpes, influenza, pertussis, meningitis, etc.

Tips & Tricks

- Polymerase Chain Reaction (PCR) was developed by Kary Mullis in 1983 and got Nobel prize for chemistry.
- Recombinant DNA is also called chimeric DNA.
- Delayed ripening is possible by reducing the amount of cell wall degrading enzyme 'Polygalacturonase' responsible for fruit softening.

Ordinary Thinking

Objective Questions

Recombinant DNA Technology

1. The linking of antibiotic resistance gene with the plasmid vector became possible with **[CBSE PMT 2008]**
   (a) DNA polymerase (b) Exonucleases
   (c) DNA ligase (d) Endonucleases

2. Main objective of production./use of herbicide resistant GM crops is to **[CBSE PMT 2008]**
   (a) Encourage eco-friendly herbicides
   (b) Reduce herbicide accumulation in food articles for health safety
   (c) Eliminate weeds from the field without the use of manual labour
   (d) Eliminate weeds from the field without the use of herbicides

3. Which of these is used as vector in gene therapy for SCID **[Kerala PMT 2009]**

   Or

   Which of the following has the ability to transform normal cells into cancerous cell in animal **[NCERT]**
   (a) Arbovirus (b) Rotavirus
   (c) Enterovirus (d) Parvovirus
   (e) Retrovirus

4. Which one among the following is just a cloning plasmid not an expression plasmid **[WB JEE 2008]**
   (a) pBAD–18–Cam (b) pBCSK
   (c) pUC18 (d) pET

5. Branch dealing with genetic engineering is **[MP PMT 1993; BHU 2006]**
   (a) Eugenics (b) Euthenics
   (c) Euphenics (d) None of these

6. Genetic engineering means **[CMC Vellore 1993]**
   (a) Manipulation of cell contents
   (b) Test tube babies
   (c) Manipulation of cytochromes
   (d) Manipulation (modification) of genes

7. Who among the following scientists is associated with the discoveries in genetic engineering **[CBSE PMT 1993]**
   (a) Khorana (b) Watson
   (c) Crick (d) Messelson

8. It is now possible to breed plants and animals with desired characters through **[KCET 1994; BHU 2006]**
   (a) Genetic engineering
   (b) Chromosome engineering
   (c) Ikebana technique
   (d) Tissue culture

9. Which of the following organelles is related with genetic engineering/gene cloning **[NCERT; CBSE PMT 1994, 99; AFMC 1997, 99; AIIMS 2001; DPMT 2003; BVP 2004; CBSE PMT (Mains) 2010]**
   (a) Golgi apparatus (b) Lysosomes
   (c) Mitochondria (d) Plasmids

10. In genetic engineering, a DNA segment (gene) of interest, is transferred to the host cell through a vector. Consider the following four agents (A-D) in this regard and select the correct option about which one or more of these can be used as a vector/vectors

    Statements

    (A) A bacterium (B) Plasmid
    (C) Plasmodium (D) Bacteriophage

    Options **[CBSE PMT (Mains) 2010]**
    (a) (A), (B) and (D) only (b) (A) only
    (c) (A) and (C) only (d) (B) and (D) only

11. Recombinant DNA (rDNA) technology is related with **[NCERT; BHU 1999; Kerala PMT 2007]**
    (a) C. Darwin (b) Stanley Cohen
    (c) Herbert Boyer (d) Both (b) and (c)

12. A desirable change in genotype of an organism is obtained by **[MHCET 2015]**
    (a) DNA replication (b) Protein synthesis
    (c) rDNA technology (d) m-RNA formation

13. Which of these is widely used in genetic engineering **[CPMT 2003]**
    (a) Anopheles (b) Dragon fly
    (c) Dragon lizard (d) Fruit fly

14. Identify the plasmid **[KCET 2004; Kerala PMT 2010]**
    (a) AIU I (b) Hind III
    (c) Eco RI (d) pBr 322

15. In recombination vector used is **[BCECE 2005; MP PMT 2007]**
    (a) Protein
    (b) *Agrobacterium tumefaciens*
    (c) Nucleic acid
    (d) Cellulose

16. First biochemical to be produced commercially by microbial cloning and genetic engineering is **[BHU 2005]**
    (a) Human insulin (b) Penicillin
    (c) Interferons (d) Fertility factors

17. Which of the following option is correct for recombinant DNA technology **[GUJCET 2014]**
    (a) Exonuclease enzyme removes nucleotides from site within DNA
    (b) Endonuclease enzyme removes nucleotides from the ends of DNA
    (c) Endonuclease enzyme cut long polandric DNA strand
    (d) Exonuclease enzyme removes nucleotides from ends of DNA

18. Restriction endonucleases are most widely used in recombinant DNA technology. They are obtained from **[KCET 2006]**
    (a) Bacteriophages (b) Bacterial cells
    (c) Plasmids (d) All prokaryotic cells

19. In recombinant DNA technique the term vector refers to **[Odisha JEE 2010]**
    (a) Plasmids that can transfer foreign DNA into a living cell
    (b) Cosmids that can cut DNA at specific base sequence
    (c) Plasmids that can join different DNA fragments
    (d) Cosmids that can degrade harmful proteins

20. An analysis of chromosomal DNA using the southern hybridization technique **does not** use **[CBSE PMT 2014]**
(a) Autoradiography (b) PCR
(c) Electrophoresis (d) Blotting

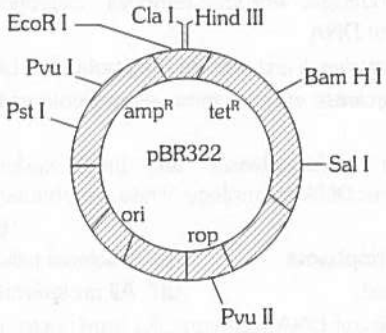
21. Genetic engineering has been successfully used for producing **[CBSE PMT (Pre.) 2010]**
(a) Animals like bulls for farm work as they have super power
(b) Transgenic mice for testing safety of polio vaccine before use in humans
(c) Transgenic models for studying new treatments for certain cardiac diseases
(d) Transgenic Cow-Rosie which produces high fat milk for making ghee

22. pBR_{322}, which is frequently used as a vector for cloning gene in *E. coli* is *a/an* **[AIIMS 2010]**
(a) Original bacterial plasmid
(b) Modified bacterial plasmid
(c) Viral genome
(d) Transposon

23. Which one of the following techniques made it possible to genetically engineer living organisms
[MP PMT 2003; CBSE PMT (Mains) 2011]
Or
The experimental manipulation of DNA of different species producing recombinant DNA is known as **[NCERT]**
(a) Heavier isotope labeling
(b) Hybridization
(c) Recombinant DNA techniques
(d) X-ray diffraction

24. The enzymes which are absolutely necessary for recombinant DNA technology are **[KCET 2012]**
(a) Restriction endonucleases and topoisomerases
(b) Endonucleases and polymerases
(c) Restriction endonucleases and ligases
(d) Peptidases and ligases

25. The figure below is the diagrammatic representation of the *E. coli* vector *pBR* 322. Which one of the given options correctly identifies its certain component (s)



[NCERT; CBSE PMT (Pre.) 2012]
(a) Ori-original restriction enzyme
(b) Rop-reduced osmotic pressure
(c) Hind III, *EcoRI* – selectable markers
(d) amp^R, tet^R – Antibiotic resistance genes

26. *PCR* and Restriction Fragment Length Polymorphism are the methods for **[NCERT; CBSE PMT (Pre.) 2012]**
(a) Study of enzymes (b) Genetic transformation
(c) *DNA* sequencing (d) Genetic-Fingerprinting

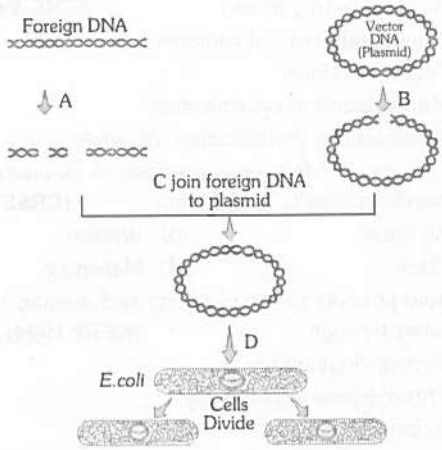
27. Fearing that the child to be born may have a genetic disorder, a couple goes to a doctor. Which one of the following techniques is likely to be suggested by the doctor to cure the genetic disorder **[KCET 2012]**
Or
Which kind of therapy was given in 1990 to a four year old girl with adenosine deaminase (ADA) deficiency **[NEET (Phase-II) 2016]**
(a) Hybridoma technology (b) Gene therapy
(c) *r*DNA technology (d) Embryo transfer

28. In genetic engineering, the antibiotics are used **[NCERT; CBSE PMT (Mains) 2012]**
(a) As selectable markers
(b) To select healthy vectors
(c) As sequences from where replication starts
(d) To keep the cultures free of infection

29. The colonies of recombinant bacteria appear white in contrast to blue colonies of non-recombinant bacteria because of **[NEET 2013]**
(a) Inactivation of glycosidase enzyme in recombinant bacteria
(b) Non-recombinant bacteria containing beta-galactosidase
(c) Insertional inactivation of alpha-galactosidase in non-recombinant bacteria
(d) Insertional inactivation of alpha-galactosidase in recombinant bacteria

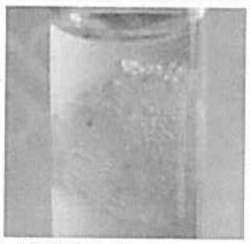
30. During the process of isolation of DNA, chilled ethanol is added to **[NCERT; NEET (Karnataka) 2013]**
(a) Precipitate DNA
(b) Break open the cell to release DNA
(c) Facilitate action of restriction enzymes
(d) Remove proteins such as histones

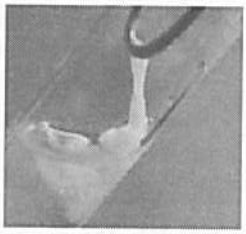
31. The below figure refers to recombinant DNA technology. Identify A, B, C and D respectively **[NCERT]**



	A	B	C	D
(a)	Restriction Endonuclease	Restriction Endonuclease	DNA ligase	Transformation
(b)	Exonuclease	Endonuclease	Hydrolase	Transduction
(c)	Endonuclease	Exonuclease	DNA ligase	Transformation
(d)	Exonuclease	Endonuclease	DNA ligase	Transformation

32. When the chilled ethanol is added in purified DNA, it ultimately precipitates out. This can be shown in the figure as collection of fine threads in the suspension. This process is known as **[NCERT]**





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

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

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

35. Which of the following restriction enzymes produces blunt ends **[NEET (Phase-II) 2016]**
(a) *Hind* III (b) *Sal I*
(c) *Eco* RV (d) *Xho* I

36. A gene whose expression helps to identify transformed cell is known as **[NEET 2017]**
(a) Selectable marker (b) Vector
(c) Plasmid (d) Structural gene

NCERT Exemplar Questions

1. Rising of dough is due to **[NCERT]**
(a) Multiplication of yeast
(b) Production of CO_2
(c) Emulsification
(d) Hydrolysis of wheat flour starch into sugars

2. Which of the following enzymes catalyse the removal of nucleotides from the ends of DNA **[NCERT]**
(a) Endonuclease (b) Exonuclease
(c) DNA ligase (d) Hind – II

3. The transfer of genetic material from one bacterium to another through the mediation of a viral vector is termed as **[NCERT]**
(a) Transduction (b) Conjugation
(c) Transformation (d) Translation

4. Which of the given statements is correct in the context of visualizing DNA molecules separated by agarose gel electrophoresis **[NCERT; NEET 2017]**
(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

5. 'Restriction' in Restriction enzyme refers to **[NCERT]**
(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

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

7. Which of the following statements does not hold true for restriction enzyme **[NCERT]**
(a) It recognizes 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

8. The most important feature in a plasmid to serve as a vector in gene cloning experiment is **[NCERT]**
(a) Origin of replication (ori)
(b) Presence of a selectable marker
(c) Presence of sites for restriction endonuclease
(d) Its size

9. While isolating DNA from bacteria, which of the following enzymes is not required **[NCERT]**
(a) Lysozyme (b) Ribonuclease
(c) Deoxyribonuclease (d) Protease

10. Which of the following contributed in popularizing the PCR (polymerase chain reactions) technique **[NCERT]**
(a) Easy availability of DNA template
(b) Availability of synthetic primers
(c) Availability of cheap deoxyribonucleotides
(d) Availability of 'Thermostable' DNA polymerase

11. An antibiotic resistance gene in a vector usually helps in the selection of **[NCERT]**
(a) Competent bacterial cells
(b) Transformed bacterial cells
(c) Recombinant bacterial cells
(d) None of the above

12. Significance of 'hear shock' method in bacterial transformation is to facilitate **[NCERT]**
(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

13. The role of DNA ligase in the construction of a recombinant DNA molecule is **[NCERT]**
(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

14. Which of the following bacteria is not a source of restriction endonuclease **[NCERT]**
(a) *Haemophilus influenzae* (b) *Escherichia coli*
(c) *Entamoeba coli* (d) *Bacillus amyloliquefaciens*

15. Which of the following steps are catalysed by Taq DNA polymerase in a PCR reaction [NCERT]
(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

16. 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 [NCERT]
(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

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

18. Who among the following was awarded the Nobel Prize for the development of PCR technique [NCERT]
(a) Herbert Boyer (b) Hargovind Khurana
(c) Kary Mullis (d) Arthur Kornberg

Critical Thinking

Objective Questions

1. During DNA purification, which enzyme is used to treat the plant cell
(a) Ribonuclease (b) Cellulase
(c) Chitinase (d) Ligase

2. The stirred-tank reactor is usually
(a) Cylindrical (b) Rounded
(c) Cup-shaped (d) Flattened

3. A bioreactor refers to [NCERT]
(a) A device in which substances are treated to stimulate biochemical transformation by living cells
(b) A nuclear reactor for biological studies
(c) A tank for biochemical reactions
(d) Organisms badly reacting to stimuli

4. Chimeric DNA is
(a) A part of recombinant DNA
(b) In fact passenger DNA
(c) Recombinant DNA formed by combining vector DNA and Passenger DNA
(d) Residual DNA that has no role in genetic engineering

5. Which one of the following correctly explains the term 'chimera'
(a) Spontaneously induced deletions
(b) Breaking a part of chromosome segment during mutation
(c) Development of genetically diverse tissues in the same organism
(d) During mutation at segregation, chromosome become equally distributed

6. Why is recombinant DNA (rDNA) technology called genetic engineering
(a) It involves sophisticated technology at microscopic level
(b) Knowledge of engineering is must in rDNA technology
(c) It involves manipulation of two DNAs
(d) It includes an authorized degree in engineering

7. In rDNA technology, in order to make the bacterial host cells 'competent' to accept the rDNA, these are kept in
(a) Dilute solution of *CsCl*
(b) Divalent anions such as phosphates
(c) Chilled ethanol
(d) Divalent cations such as calcium

8. Which of the following statement is correct in the context of observing DNA fragments 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

9. Recombinant DNA is forced to enter the host cells by incubating the cells with rDNA first
(a) On ice followed by heat shock and then again on ice
(b) At low temperature followed by heat shock and then on ice
(c) In ethidium bromide followed by calcium salts
(d) Into chilled ethanol followed by ice and then into *CsCl*

10. The desired product of rDNA technology produced through bioreactors on large scale undergoes
(a) Elution (b) Enzymatic action
(c) Biomonitoring (d) Downstream processing

11. The cloning vector M13 has genetic material [MHCET 2015]
(a) ssRNA (b) dsRNA
(c) ssDNA (d) dsDNA

12. Match Column I and Column II and select the right option given below [NCERT]

Column I		Column II	
I.	Recombinant DNA technology	A.	Vector
II.	Cloning Vehicles	B.	Sealing enzyme
III.	Macromolecular Separation	C.	Electrophoresis
IV.	DNA Ligase	D.	Genetic engineering

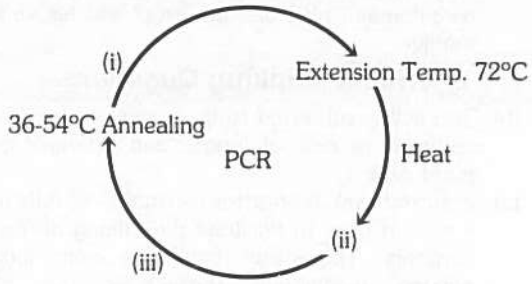
(a) I – D, II – A, III – B, IV – C (b) I – A, II – D, III – B, IV – C
(c) I – D, II – A, III – C, IV – B (d) I – B, II – A, III – D, IV – C

13. The function of a selectable maker is [KCET 2015]
(a) Eliminating transformants and permitting non-transformants
(b) Identify ori site
(c) Elimination of non-transformants and permitting transformants
(d) To destroy recognition sites

14. Which of the following is/are used in recombinant DNA technology [NCERT]
A. Agarose gel B. Ethidium bromide
C. Plasmid vector D. Restriction endonuclease
(a) A, B (b) B, C
(c) C, D (d) A, B, C, D

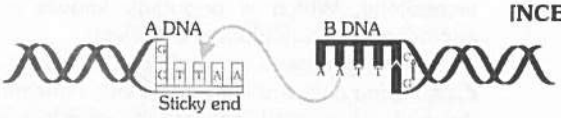
15. DNA recombinant technology uses
A. Restriction endonucleases B. DNA ligase
C. Cloning vector D. Electrophoresis
(a) A, B (b) B, C
(c) C, D (d) A, B, C, D

16. The following cycle refer to the PCR process. Name the factors or steps indicated with numbers **[NCERT]**



(a) (i) Taq polymerase (ii) Extension (iii) Ligation
(b) (i) Primer (ii) Denaturation at 94°C (iii) Taq polymerase
(c) (i) Denaturation at 94°C (ii) Taq polymerase (iii) Primer
(d) (i) Taq polymerase (ii) Denaturation at 94°C (iii) Primer

17. Observe the figure and select the correct option out of (a - d) **[NCERT]**



	A DNA	B DNA	Enzyme recognizing palindrome	Enzyme joining the sticky ends
(a)	Vector	Foreign	DNA ligase	Exonuclease
(b)	Vector	Foreign	Exonuclease	DNA ligase
(c)	Vector	Foreign	EcoRI	DNA ligase
(d)	Vector	Foreign	DNA ligase	EcoRI

18. The first human hormone drug produced by recombinant DNA technology genetic engineering is **[Manipal 2005; CBSE PMT 2014]**

Or

Genetically engineered bacteria are being employed for production of **[WB JEE 2009, AFMC 2010]**
(a) Thyroxin (b) Progesterone
(c) Insulin (d) Estrogen

19. Which vector can clone only a small fragment of DNA **[CBSE PMT 2014; KCET 2015]**
(a) Plasmid
(b) Cosmid
(c) Bacterial artificial chromosome
(d) Yeast artificial chromosome

20. Which of the following is not a component of downstream processing **[NEET (Phase-II) 2016]**
(a) Expression
(b) Separation
(c) Purification
(d) Preservation

21. The process of separation and purification of expressed protein before marketing is called **[NEET 2017]**
(a) Upstream processing
(b) Downstream processing
(c) Bioprocessing
(d) Postproduction processing

A R Assertion & Reason

Read the assertion and reason carefully to mark the correct option out of the options given below :

(a) If both the assertion and the reason are true and the reason is a correct explanation of the assertion
(b) If both the assertion and reason are true but the reason is not a correct explanation of the assertion
(c) If the assertion is true but the reason is false
(d) If both the assertion and reason are false
(e) If the assertion is false but reason is true

1. 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 population which express the desired gene. **[AIIMS 2005, 08]**

2. Assertion : Plasmids are extrachromosomal DNA.
Reason : Plasmids are found in bacteria and are useful in genetic engineering. **[AIIMS 2001]**

3. Assertion : Plasmids are single stranded extra chromosomal DNA.
Reason : Plasmids are found in Eukaryotic cells. **[AIIMS 1997, 2002, 13]**

4. Assertion : Recognition site should be preferably single and responsive to commonly used restriction enzyme.
Reason : In *pBR* 322 alien DNA is ligated generally in the area of *Bam-HI* site of tetracycline resistance gene. **[AIIMS 2009]**

5. Assertion : A gene from *Bacillus thuringiensis* is incorporated in plant genome to increase yield.
Reason : It is Bt toxin producing gene which kills larvae of insects. **[AIIMS 2011]**

Answers

Recombinant DNA Technology

1	c	2	b	3	e	4	c	5	c
6	d	7	a	8	a	9	d	10	d
11	d	12	c	13	d	14	d	15	b
16	a	17	d	18	b	19	a	20	a
21	b	22	b	23	c	24	c	25	d
26	d	27	b	28	a	29	b	30	a
31	a	32	d	33	d	34	d	35	c
36	a								

NCERT Exemplar Questions

1	b	2	b	3	a	4	d	5	c
6	d	7	c	8	a	9	c	10	d

11	b	12	c	13	a	14	c	15	c
16	a	17	c	18	c				

Critical Thinking Questions

1	b	2	a	3	a	4	c	5	c
6	c	7	d	8	d	9	a	10	d
11	c	12	c	13	c	14	d	15	d
16	d	17	c	18	c	19	a	20	a
21	b								

Assertion and Reason

1	a	2	a	3	d	4	b	5	a

Answers and Solutions

Recombinant DNA Technology

6. (d) Genetic engineering is DNA manipulation technology that produces and modifies DNA sequences for creating cells, tissues organs and individuals to suit human requirements.

7. (a) *Hargovind Khorana* is associated with genetic engineering. He synthesized 'gene' artificially' in a test tube (1969).

9. (d) Plasmids are extrachromosomal covalently closed circular double stranded molecules of DNA present in most prokaryotes. Therefore they are used as a vector in genetic engineering.

10. (d) Plasmids and bacteriophages are used as vectors in genetic engineering.

11. (d) The first recombinant DNA was constructed by Stanley cohen and Herbert in 1972. They cut the piece of DNA from a plasmid carrying antibiotic-resistance gene in the bacterium *Salmonella typhimurium* and linked it to the plasmid of *Escherichia coli*.

13. (d) Fruit fly also known as drosophila is widely used in genetic engineering.

14. (d) Plasmid *pBr* 322 was one of the first widely used cloning vectors, it contains both ampicillin and tetracycline resistance genes.

16. (a) Commercially production of *E.coli* genetically engineered human insulin.

22. (b) *E.* coli plasmid pBR_{322} is the most versatile and widely used cloning vector, it contains both ampicillin and tetracyclin-resistance genes and a number of unique restriction enzyme cleavage sites.

25. (d) In *pBR*322 ori-represents site of origin or replication, rop-represents those proteins that take part in replication of plasmid. Hind III, *ECORI*- Recoginition sites of Restriction endonucleases, amp^R and tet^R - They are antibiotic resistant gene part.

26. (d) *PCR* is used in amplification of *DNA* segment and used in genetic fingerprinting.

33. (d) Plasmids are extrachromosomal double stranded circular DNA.

34. (d) The exonuclease enzyme serves as molecular scissor and catalyzing removal of nucleotides from ends of DNA.

35. (c) Eco RV has restriction sequence –
5' – GAT ATC – 3'
3' – CTA TAG – 5'

36. (a) Selectable marker genes help to separate transformant from non transformant and recombinant from non recombinant. pBR 322 has amp^r and tet^r as selectable markers

Critical Thinking Questions

1. (b) The cell is subjected to lysozyme in case of bacteria, chitinase in case of fungus and cellulose in case of plant cells.

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

5. (c) Chimera is a mixture of genetically different tissues (in an organ or structure).

6. (c) The principle of biotechnology (BT) is based on a set of molecular techniques collectively called r-DNA technology. Which is popularly known as genetic engineering (manipulation of genes).

7. (d) The cloning organisms or bacterial host cells (e.g. *Escheriohia coli*) are kept in specific concentration of divalent cations such as Ca^{2+} in order to make them more permeable and 'Competent' for rDNA.

8. (d) These DNA fragments according to their size and can be seen only after staining with a compound called ethidium bromide followed by ultraviolet radiation exposure.

9. (a) The host cells with r-DNA are first subjected to ice followed by heat shock (42^0C) and then to ice. As a result, rDNA molecules are easily introduced into the host cells. This process is called transformation.

10. (d) The desired products developed by bioreactors undergo downstream processing (Separation and Purification) in order to yield finished product which is now ready for marketing.

19. (a) Plasmid can clone only a small fragment of DNA about 10 kbp size
Cosmid – 45 kbp
YAC – 1 Mbp/1000 kbp – 2,500 kbp
BAC – 300 to 350 kbp.

20. (a) Expression of recombinant DNA is parts of upstream processing

Assertion and Reason

1. (a) Bacteria and yeast can easily grow in culture medium and multiply very fast so it is best for making many copies of recombinant DNA, and express character of desired gene.

2. (a) Plasmids are possessed by bacteria. These are extrachromosomal DNA. These are used in genetic engineering

3. (d) Plasmids are extrachromosomal double stranded small circular DNA usually found in bacterial cell in additon to main genetic material.

4. (b) Recognition site or site where alien DNA can be inserted should be single because occurrence of more than one recognition site will cause DNA fragmentation and disturb gene cloning.

5. (a)

UNIVERSAL BOOK DEPOT 1960

Biotechnology : Principles and Processes

SET Self Evaluation Test

1. Which of the following tools of recombinant DNA technology is incorrectly paired with its use **[NCERT]**

(a) Restriction enzyme-production of RFLPs

(b) DNA ligase-enzyme that cuts DNA, creating the stickly ends of restriction fragments

(c) DNA polymerase-used in a polymerase chain reaction to amplify sections of DNA

(d) Reverse transcriptase-production of cDNA from mRNA

2. In recombinant DNA methods, the term vector refers to

(a) The enzyme that cuts DNA into restriction fragments

(b) The sticky end of a DNA fragment

(c) A plasmid used to transfer DNA into a living cell

(d) A DNA probe used to identify a particular gene

3. Identify the labelled items A, B, C, D, E, F and G in the diagram below from the list-I to VII given with

Components –

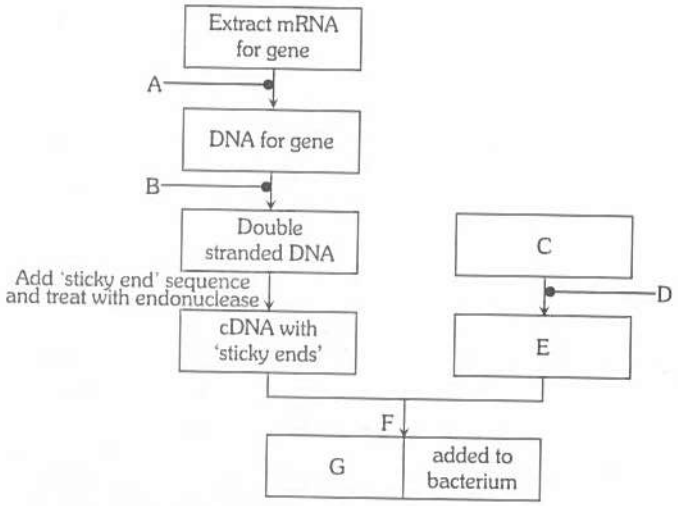
I. DNA polymerase II. Plasmid

III. Plasmid with 'sticky ends' IV. DNA ligase

V. Restriction endonuclease VI. Recombinant DNA

VII. Reverse transcriptase

The correct components are –



	A	B	C	D	E	F	G
(a)	VII	I	II	V	III	IV	VI
(b)	VII	VI	V	IV	III	II	I
(c)	VII	V	III	I	II	IV	VI
(d)	I	II	IV	VI	III	V	VII

4. When a recombinant DNA is inserted within the coding sequence of an enzyme, β – galactosidase **[NCERT]**

(a) This result into inactivation of the enzyme

(b) This is called insertional inactivation

(c) In the presence of insertion, the colonies do not produce any colour

(d) All of these

5. If recombinant DNA is inserted within the coding sequence of enzyme galactosidase, which of the following will occur in case of non-recombinants **[NCERT]**

(a) Insertional inactivation

(b) Colonies do not produce any colour

(c) Chromogenic substrate gives blue colour

(d) Inactivation of enzyme galactosidase

6. Following enzymes/techniques are used in the process of recombinant DNA technology

A. EcoRI to cut the isolated genome

B. DNA ligase

C. Protease and ribonuclease for removal of proteins and RNA from DNA

D. Production of recombinant hosts

E. Lysozyme for isolation of the genetic material (DNA)

F. Gel electrophoresis for separation and isolation of DNA fragments

Mark the correct sequence of their use **[NCERT]**

(a) C, E, B, F, A, D (b) E, C, A, B, F, D

(c) E, C, A, F, B, D (d) A, E, C, B, D, F

7. I. Recombinant DNA technology is used to improve crop plants by increasing their productivity, by making them more nutritious and by developing disease resistant

II. Bt cotton is resistant to bollworm infestation

III. *Bacillus thuringiensis* form Cry protein during any phase of their growth

IV. *Bacillus thuringiensis* is not harmed by self Cry protein because of its occurrence as protoxin (inactive)

V. Protoxin Cry protein is changed into active Cry protein in the stomach of insects due to alkaline pH in stomach

(a) All are correct (b) I and IV are correct

(c) Only III is false (d) All are false

8. DNA polymerase enzyme is isolated from which bacteria **[GUJCET 2015]**

(a) *E. Coli* (b) *Thermus aquaticus*

(c) *Bacillus thuringenesis* (d) *Agro bacterium*

9. Which of the following techniques serve the purpose of early diagnosis

I. r-DNA technology
II. PCR
III. ELISA
IV. Convential method of diagnosis (serum, urine analysis, etc)

(a) I, II, III (b) IV only
(c) III only (d) All

10. The DNA molecule to which the gene of interest is integrated for cloning is called **[AIPMT 2015]**

(a) Vector
(b) Template
(c) Carrier
(d) Transformer

11. The application of microbial metabolism to transform simple raw materials into valuable products is **[MP PMT 1993]**

(a) Biocatalysis
(b) Genetic engineering
(c) Tissue culture
(d) Fermentation

12. For rapid production of alcohol, immobilised yeast cells are kept in

(a) Silica gel
(b) Wire netting
(c) Porcelain columns
(d) Calcium alginate beads

13. What is the source of EcoRI **[AIIMS 2011]**

(a) *Escherichia coli* RI
(b) *Escherichia coli* RI 13
(c) *Escherichia coli* RX 13
(d) *Escherichia coli* RY 13

Answers

1	b	2	c	3	a	4	d	5	c
6	c	7	c	8	b	9	a	10	a
11	d	12	d	13	d				

* * *