XII
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BIOTECHNOLOGY- PRINCIPLES AND PROCESS

SYLLABUS

Principles and process of Biotechnology: Genetic engineering (Recombinant DNA technology).

KEY CONCEPTS

BIOTECHNOLOGY

* Biotechnology may be defined as use of micro-organism, animals, or plant cells or their products to generate different products at industrial scale and services useful to human beings.
* Old biotechnology are based on the natural capabilities of micro organisms. e.g. formation of citricacid, production of penicillin by penicillium Notatum.
* New biotechnology is based on Recombinant DNA technology. e.g. Human gene producing Insulin has been transferred and expressed in bacteria like E.coli.
* The definition given by EFB (European Federation of Biotechnology) : 'The integration of natural science and organisms, cells, parts thereof, and molecular analogues for products and services'.
* The term Biotechnology was given by **Karl Ereky.**
* **Paul Bergh** (Father of genetic engineering) transferred gene of SV 40 virus (simian virus) in to E.coli with the help of λ - phage. (Nobel prize - 1980)
* The concept of genetic engineering was the outcome of two very significant discoveries made in bacterial research. These were:

(i) Presence of extrachromosomal DNA fragments called **plasmids** in the bacterial cell, which replicate along with chromosomal DNA of the bacterium.

(ii) Presence of enzymes restriction endonucleases which cut DNA at specific sites. These enzymes are, therefore, called **'molecular scissors'.**

PRINCIPLES OF BIOTECHNOLOGY

* Two core techniques that enabled birth of modern biotechnology:

(i) **Genetic engineering:** Techniques to alter the chemistry of genetic material (DNA and RNA) to introduce into host organisms and thus change the phenotype of the host organism.

Bioprocess engineering

Maintenance of sterile (microbial contamination-free) ambient chemical engineering processes to enable growth of only the desired microbe/eukaryotic cell in large quantities.

(ii) **Genetic engineering:** "Recombinant DNA technology" or also called "Genetic Engineering" deals about, the production of new combinations of genetic material (artificially) in the laboratory. These "recombinant DNA" (rDNA) molecules are then introduced into host cells, where they can be propagated and multiplied.

* Three basic steps in genetically modifying an organism
  - Identification of DNA with desirable gene.
  - Introduction of the identified DNA into the host.

- Maintenance of introduced DNA in the host and transfer of the DNA to its progeny.

* In 1972, **Stanley Cohen and Herbert Boyer** constructed the first recombinant DNA.
* Steps carried out in constructing first recombinant DNA.

- The construction of the first recombinant DNA emerged from the possibility of linking a gene encoding antibiotic resistance with a native Plasmid of *Salmonella typhimurium*.
- The cutting of DNA at specific locations became possible with the discovery of the so called 'Molecular scissors" - restriction enzymes. The cut piece of DNA was then linked with the plasmid DNA with the help of another enzyme called **DNA ligase**. These plasmid DNA act as **vectors** to transfer the piece of DNA attached to it. A plasmid can be used as vector to deliver an alien piece of DNA into the host organism.

TOOLS OF RECOMBINANT DNA TECHNOLOGY

Restriction Enzymes (Molecular Scissors):

* Cleave DNA duplex at specific points in such a way that they come to posses short single stranded free ends.
* In the year 1963 two enzymes discovered from ***Escherichia coli*** which restrict the growth of bacteriophage in it.
  One of these added methyl groups to DNA.
  Other cut the phage DNA. (restriction endonuclease)
* The first restriction endonuclease discovered is *Hind II*.(Isolated by Smith,Wilcox and Kelley from *Haemophilus influenza* bacterium)
* *Hind II* always cut DNA molecule at particular point by recognizing a specific sequence of six base pairs. This is called **recognition sequence** for *Hind II*.

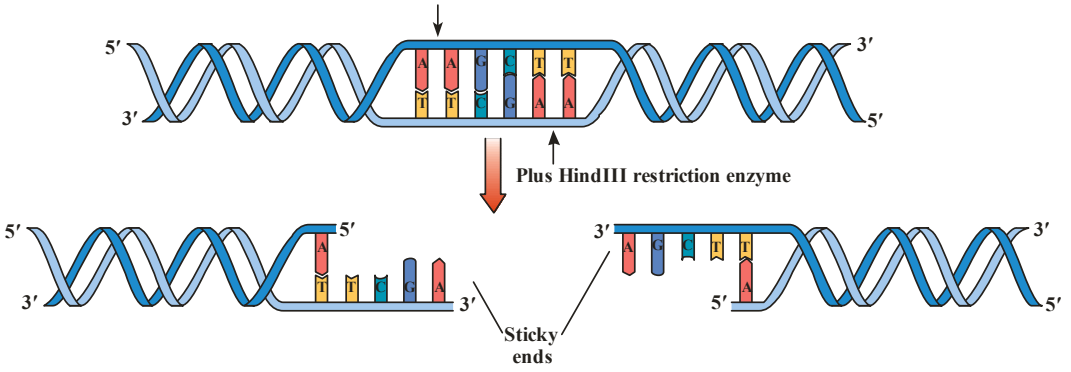


Figure : Cutting DNA with a restriction enzyme
Many restriction enzymes, such as Hind III, cut DNA at sequences that are palindromic, producing complementary sticky ends. The small black arrows designate the enzyme's cleavage sites.

* **Examples of Restriction enzyme :**

Recognition sequences of some restriction endonucleases

Name	Recognition sequence	End after cleavage		Source
Eco RI	–G A A T T C– –C T T A A G–	–G –C T T A A	A A T T C– G–	*Escherichia coli*-containing drug resistant plasmid RI
Hind III	–A A G C T T– –T T C G A A–	–A –T T C G A	A G C T T– A–	*Haemophilus influenzae*
Bam HI	–G G A T C C– –C C T A G G–	–G –C C T A G	G A T C C– G–	*Bacillus amyloliquefaciens*
Hae III	–G G C C– –C C G G–	–G G –C C	C C– G G–	*Haemophilus aegyptius*

Convention for naming restriction endonuclease:

* The first letter of the name comes from the genus.
* Second two letters come from the species of the prokaryotic cell from which the enzyme isolated
* The fourth letter is in capital form derived from the Strain of microbes.
* The Roman letter followed is the order of discovery
* Example: EcoRI comes from Escherichia coli RY 13, Hind II from *Haemophilus influenza* Rd, *Bam* H I from *Bacillus amyloliquefaciens* H, *Eco* R II from *E. Coli* R 245.

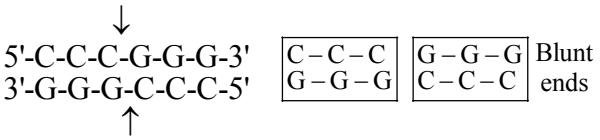
Mechanism

* Restriction enzyme belongs to nucleases. There are two kind of nucleases:
  - **Exonuclease** removes nucleotides from the free ends of the DNA.
  - **Endonucleases** make cuts at specific positions within the DNA.
* Each restriction endonuclease recognizes a specific palindromic nucleotide sequences in the DNA.
* **Palindromes** are the group of letters that read same both forward and backward, e.g. "MALAYALAM".
* The palindrome in DNA is a sequence of base pairs that reads same on the two strands when orientation of reading is kept same.

  5' ____ G A A T T C ____ 3'

  3' ____ C T T A A G ____ 5'
* **Blunt or flush ends** are produced by many restriction enzymes which cleave both stands of DNA at exactly the same nucleotide position, in the centre of recognition site.

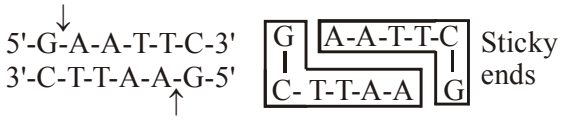
  For example, *Sma I* recognises 6 nucleotide palindromic sequence.



It cuts both DNA strands producing blunt ends.

* **Sticky or cohesive ends** are produced when restriction enzymes do not cut DNA at the same nucleotide position but cut the recognition sequence unequally. This produces short, single-stranded overhangs at each end. These are known as sticky ends.

  For example, *Eco RI* recognises 6 nucleotide palindromic sequence.



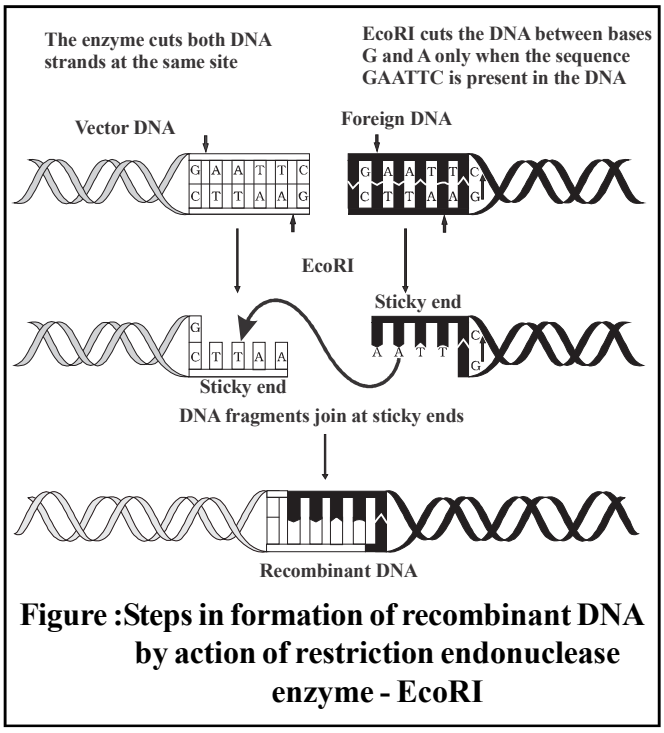


Figure :Steps in formation of recombinant DNA by action of restriction endonuclease enzyme - EcoRI

* This stickiness of the ends facilitates the action of the enzyme DNA ligases.
* The foreign DNA and the host DNA cut by the same restriction endonuclease, the resultant DNA fragments have the same kind of 'sticky-ends' and these can be joined together using **DNA ligases.**

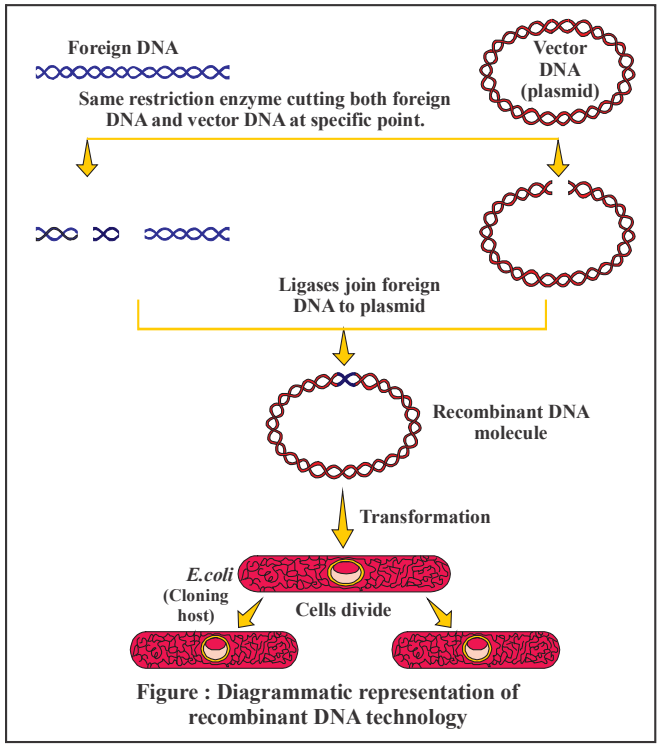


Figure : Diagrammatic representation of recombinant DNA technology

Other Enzymes used in Recombinant DNA Technology

(a) DNA Ligase :

* This enzyme forms phospho-diester bonds between adjacent nucleotides and covalently links two individual fragments of double-stranded DNA.
* The action of the ligase enzyme requires a phosphate group at the 5' carbon of one nucleotide and a hydroxyl group at the 3' carbon of the adjacent nucleotide to form the phosphodiester bond between these two nucleotides.
* The enzyme used most often in the rDNA technology is T_4 DNA ligase, which is encoded by phage T_4.

(b) Alkaline Phosphatase (AP) :

* As mentioned above, ligation absolutely requires the presence of 5' phosphate group at the DNA site to be ligated. If this phosphate group is removed, this DNA cannot be ligated.
* The enzyme alkaline phosphatase is used to remove the phosphate group from the 5' end of a DNA molecule, leaving a free 5' hydroxyl group.
* This enzyme can be isolated from bacteria (BAP) or calf intestine (CAP).
* It is used to prevent unwanted self-ligation of vector DNA molecules in procedures of rDNA technology.
* However, ligation of the vector to the insert can occur as the insert still has its 5' phosphate.

(c) DNA Polymerase :

* DNA Pol I enzyme polymerizes the DNA synthesis on DNA template or complementary DNA (cDNA).
* It also catalyses a 5' $\rightarrow$ 3' and 3' $\rightarrow$ 5' exonucleolytic degradation of DNA.
* The other two enzymes are DNA polymerase II (DNA pol II) and DNA polymerase III (DNA pol III). These have almost similar catalytic activity.
* DNA pol III is about several times more active than the other two. Where there is preformed DNA template, it produces a parallel strand in the presence of ATP.

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**.
* The DNA fragments are separated according to their size through sieving effect provided by the agarose gel. Hence, the smaller the fragment size the farther it moves.

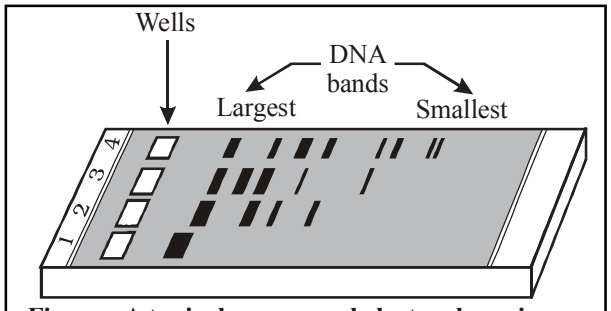


Figure : A typical agarose gel electrophoresis showing migration of undigested (lane 1) and digested set of DNA fragments (lane 2 to 4)

* The separated DNA fragments can be visualized only after staining the DNA with **Ethidium bromide** followed by exposure to UV radiation.

Now DNA fragments appear bright orange coloured bands.

* The separated bands of DNA are cut out from the **agarose** (Natural linear polymer of D-galactose and 3,6,-anhydro-L-galactose which is extracted from sea weeds) gel and extracted from the gel piece. This step is known as **Elution**.
* These DNA fragments are purified and used in constructing recombinant DNA with cloning vector.

Cloning vectors:

* Vector serves as a vehicle to carry a foreign DNA sequence into a given host cell.
* **Copy number :** It is defined as the number of copies of vectors present in a cell. It varies from 1–100 copies per cell.

Features of cloning vector:

(i) Origin of replication (Ori):

* This is the sequence where the replication starts ..... *ori* gene.
* The alien DNA linked with vector also replicates.
* Controls the copy number of the linked DNA.

(ii) Selectable marker:

* It is required to identify **recombinant** from the **non-recombinant.**
* Helps in identifying and eliminating non-transformants and selectively permitting the growth of the transformants.
* Transformation is a procedure through which a piece of foreign DNA is introduced in a host bacterium.
* Normally, the gene coding resistance to antibiotics such as ampicilin. Tetracycline, chloramphenicol or kanamycins etc are considered as useful selectable markers for *E.coli.*
* Thr normal *E.coli* cells do not carry resistance against any of antibiotics.

(iii) Cloning sites:

* In order to link the alien DNA, the vector needs to have very few, preferably single, **recognition sites (palindromic site)** for the commonly used restriction endonuclease.

(iv) Size of the vector :

* Cloning vector should be small in size. Large molecules have a tendency to breakdown during purification.

Examples of Vectors :

(a) Plasmids :

* These are extra-chromosomal, non-essential self-replicating, usually circular and double stranded DNA molecules occurring in some bacteria and also a few yeasts.
* Some of the characters carried by plasmid may not be required for normal bacterial metabolism but may be of great advantage e.g. antibiotic resistance.
* **Table : Plasmid DNA and Chromosomal DNA**

S.N.	Plasmid DNA	Chromosomal DNA
1.	This is present in prokaryotic cells (bacteria).	This is present in both prokaryotic and eukaryotic cells.
2.	This is the circular extra chromosomal DNA not associated with histone proteins.	It is linear and associated with histones proteins.
3.	It gives the cell extra characters like antibiotic resistance.	It does not provide any extra characters to the cell.

* pBR322 is one of the standard cloning vectors widely used in gene cloning experiments.

Explanation of the name pBR322 :

p-Plasmid

BR-Boliver and Rodriguez

322-Distinguishes the plasmid from others developed in the same laboratory.

Characteristics of **pBR322 :**

(i) Size = 4.3 Kb

(ii) Two sets of antibiotic resistance gene. Therefore, selection is a two step process.

(iii) Reasonably high copy number.

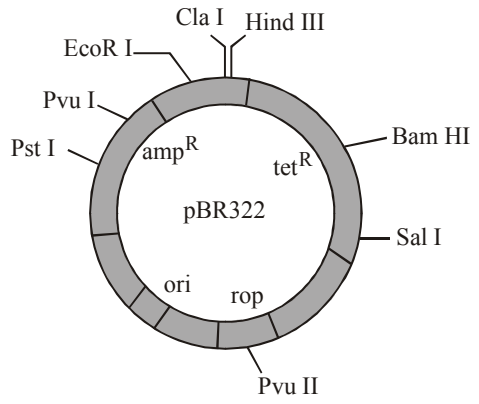


Figure : Cloning vector

E. coli cloning vector pBR322 showing restriction sites (*Hind III, EcoR I, BamH I, Sal I, Pvu II, Pst I, Cla I*), *ori* and antibiotic resistance genes (ampR and tetR). Rop codes for the proteins involved in the replication of the plasmid.

Disadvantage of pBR322

* Selection of recombinants due to inactivation of antibiotics is a cumbersome procedure because it requires simultaneous plating on two plates having different antibiotics.
* Overcoming the disadvantage of pBR322 by using another plasmid pUC8.

Advantage :

* Higher copy number (500-700).
* Identification of recombinant cells achieved by a single step i.e., plating cells onto agar medium containing amplicillin and X-gal (chromogenic substrate for β-galactosidase enzyme encoded by lac Z).

(b) Bacteriophages :

* Bacteriophages are viruses which infect bacterial cells produce new phages inside the host bacterium, and are released from the host cell to again infect other bacterial cells. M 13 and lambda (λ) phages are in common use.
* If we are able to link an alien piece of DNA with bacteriophage, we can multiply its numbers equal to the copy number of the bacteriophage.
* The plasmid and bacteriophages have the ability to replicate within bacterial cells independent of the control of chromosomal DNA.

(c) Cosmids :

* These are hybrid vectors derived from plasmids which contain *cos* site of λ phase.

(d) YAC vectors:

* Yeast artificial chromosome contain telomeric sequence, the centromere and autonomously replicating sequence from yeast chromosomes.
* These also have suitable restriction enzyme sites and genes useful as selectable markers.

(e) BAC vectors:

* Bacterial artificial chromosome is based on F plasmid (fertility) of *E. coli*.
* It contains genes for replication and maintenance of F-factor, selectable marker and cloning sites.

Passenger DNA :

* It is the DNA which is transferred from one organism into another by combining it with the vehicle DNA.
* The passenger DNA can be complementary, synthetic or random.

(i) Complementary DNA (cDNA) :

* It is synthesized on mRNA template with the help of reverse transcriptase and necessary nucleotides. The DNA strand is then separated from the hybrid RNA-DNA complex by using alkali.
* Complementary DNA strand is then synthesized over the template of cDNA with the help of DNA polymerase.
* cDNA formed through reverse transcription is shorter than the actual or in *vivo* gene because of the absence of introns or non-coding regions.

(ii) Synthetic DNA (sDNA) :

* It is synthesized with the help of DNA polymerase on DNA template.
* **Kornberg (1961)** synthesized first synthetic DNA from a mixture. of deoxyribonucleotide triphosphates, DNA polymerase enzyme, metal ions and a segment of viral DNA.
* **Khorana (1968)** synthesized first artificial gene (DNA) without a template. They synthesized the gene coding for yeast alanine t-RNA, which contained only 77 base pairs. However, it did not function in the living system. In 1979, Khorana was able to synthesize a functional tyrosine t-RNA gene of *E.coli* with 207

nucleotide pairs. Since then a number of genes have been synthesized artificially.

(iii) Random DNA : It refers to small fragments formed by breaking a chromesome with the help of restriction endonucleases.

Identification of recombinants:

(a) Inactivation of antibiotics:

If a foreign DNA ligated or inserted at the Bam H I site of tetracycline resistance gene in the vector pBR322, the recombinant plasmid will lose tetracycline resistance. (insertional inactivation).

The recombinant can be identified from the non-recombinant in following steps:

* All are grown in ampicilin medium
* One replica of above plate grown in ampicilin medium (control)
* Other replica grown in the medium containing both tetracycline and ampicilin.
* The colonies grows in plate-I but failed to grow in plate-II are identified as recombinants.

(b) Insertional inactivation

* In E.coli a plasmid called **pUC8** is used as selectable marker, which is better than pBR322.
* The foreign DNA is introduced within the coding sequence of an enzyme β-galactosidase, which convert X-Gal (chromatogenic substrate) into Galactose and 5-bromo+4 chloro indigo (blue color)
* The non-recombinant produce enzyme and give blue colored colonies.
* The recombinant unable to produce β-galactosidase and does not produce blue colored colonies after addition of chromatogenic substrate i.e. X-Gal.
* This inactivation of insertion of foreign DNA called insertional inactivation.

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

* *Agrobacterium tumefaciens*, a pathogenic bacterium of several dicot plants. This bacterium contains a plasmid called **Ti-plasmid.**(tumor inducing)
* In natural condition the *A.tumifaciens* transfer the **T-DNA** into the plant which transform normal plant cells into a tumor and direct these tumor cells to produce the chemical required by the pathogen.
* Retroviruses in animals have the ability to transform normal cells into **cancerous** cells.
* The dis-armed retroviruses are being used to transfer gene into animals.
* In Ti-plasmid the T-DNA is replaced by the gene of interest, still *A.tumifaciens* able to transfer the gene into the plant without causing tumor in plants.

Competent Host (for transformation with recombinant DNA)

* DNA is a hydrophilic molecule; it cannot pass through cell membranes.
* In order to force bacteria to take-up the plasmid, the bacterial cells must first be made 'competent' to take up DNA.
* The bacterial cell is treated with divalent cations such as calcium, which increases the efficiency of DNA up take by the bacteria.
* Recombinant DNA and the bacterial cells are incubated in ice, followed by placing them briefly at 42°C **(heat shock)** and then putting them back in ice.
* By **microinjection** the recombinant DNA directly injected into the nucleus of the animal cell.
* **Biolistics / Gene gun method**, it has been developed to introduce rDNA into mainly plant cells by using a Gene / Particle gun. In this method, microscopic particles of gold / tungsten are coated with the DNA of interest and bombarded onto cells. Disarmed Pathogen Vectors (*Agrobacterium tumefacien*s) are also used to transfer rDNA.

PROCESS OF RECOMBINANT TECHNOLOGY

* Isolation of DNA → Cutting of DNA using restriction endonuclease → Amplification of Gene using PCR → Making rDNA and insertion of it into host cell/organism → obtaining the foreign gene product Downstream processing.

Isolation of the Genetic material (DNA):

* DNA can be obtained from the cell by treating with enzymes like, **Lysozyme** for bacteria, **Cellulase** for plant cell, **Chitinase** for fungus.
* Histone protein and RNA can be removed by treating with proteases and ribonuclease
* Purified DNA ultimately precipitated by the addition of chilled ethanol.Fine threads of DNA are obtained in the suspension.

Cutting of DNA at specific location :

* The purified DNA is cut by use of restriction enzymes. Agarose gel electrophoresis used to check the progression of restriction enzymes digestion.
* DNA is a negatively charged molecule, hence it moves towards the positive electrode (anode). The process is repeated with the vector DNA also.

Amplification of Gene of Interest using PCR:

* PCR stands for **Polymerase chain reaction.** Developed by Kary Mullis.
* Multiple copy of gene of interest can be synthesized *in vitro*.
* PCR requires: DNA template, Primers (Small chemically synthesized oligonucleotides) and Enzyme.

(i) **DNA Template:** Any source that contains one or more target DNA molecules to be amplified can be taken as template.

(ii) **Primers :** Primers, which are oligo-nucleotides, usually 10-18 nucleotides long, that hybridize to the target DNA region, one to each strand of the double helix. Two primers are required and these primers are oriented with their ends facing each other, allowing synthesis of the DNA towards one another.

(iii) **Enzyme :** DNA polymerase which is stable at high temperatures (>90º) is required to carry out the synthesis of new DNA. The polymerase which is generally used in PCR reactions is known as *Taq* polymerase (isolated from a bacterium *Thermus aquaticus*). Other thermostable polymerases can also be used.

PCR includes following steps:

(a) **Denaturation:**

* Double stranded DNA made single stranded.
* It is done by heating the DNA at 94°C.
* Each single stranded DNA is called **Template strand.**

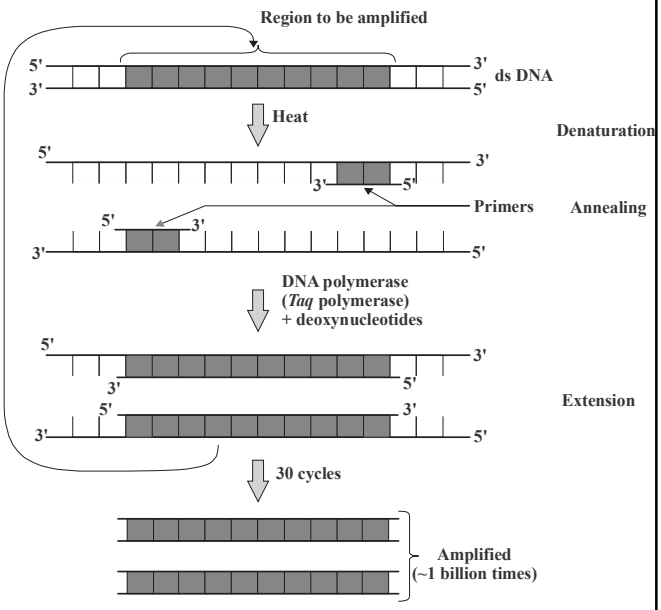


Figure : Polymerase chain reaction (PCR)

(b) **Annealing:**

* Two sets of primer (small oligonucleotide chain that are complementary to the DNA at 3' end of the DNA template) added to the medium.
* This is done at around 50°C.

(c) **Primer Extension (Polymerisation) :**

* The final step is extension, wherein *Taq* DNA polymerase (of a thermophilic bacterium *Thermus acquaticus*) synthesizes the DNA region between the primers, using dNTPs (deoxynucleoside triphosphates) and Mg^{2+}.

* It means the primers are extended towards each other so that the DNA segment lying between the two primers is copied.
* The optimum temperature for this polymerization step is 72°C.
* All steps are repeated many times to obtain several copies of desired DNA.
* From a single template molecule, it is possible to generate 2^n molecules after n number of cycles.

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 its surrounding.
* If a recombinant DNA bearing gene for resistance to an antibiotic (e.g., ampicillin) is transferred into E. coli cells, the host cells become transformed into ampicillin-resistant cells.
* If we spread the transformed cells on agar plates containing ampicillin, only transformants will grow, 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 or Recombinant product:

* The protein encoding gene is expressed in a **heterogeneous host** 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 and then purifying it by using different separation techniques.

Bioreactors :

* To produce in large quantities, the development of bioreactors, where large volume of culture can be processed, was required.
* Bioreactors can be thought of as vessels in which raw materials are biologically converted into specific products, individual enzymes, etc., using microbial plant, animal or human cells.
* A bioreactor provides the optimal conditions for achieving the desired product by providing optimum growth conditions (temperature, pH, substrate, salts, vitamins, oxygen).
* The most commonly used bioreactors are of stirring type.
* A stirred-tank reactor is usually cylindrical or with a curved base to facilitate the mixing of the reactor contents. The stirrer facilitates even mixing and oxygen availability throughout the bioreactor.

Bioreactors required following components:

(a) An agitator system
(b) An oxygen delivery system
(c) Foam control system
(d) Temperature control system
(e) pH control system
(f) Sampling ports to withdraw cultures periodically.

* **Sparged Stirred-tank reactor** is a stirred type reactor in which air is bubbled.

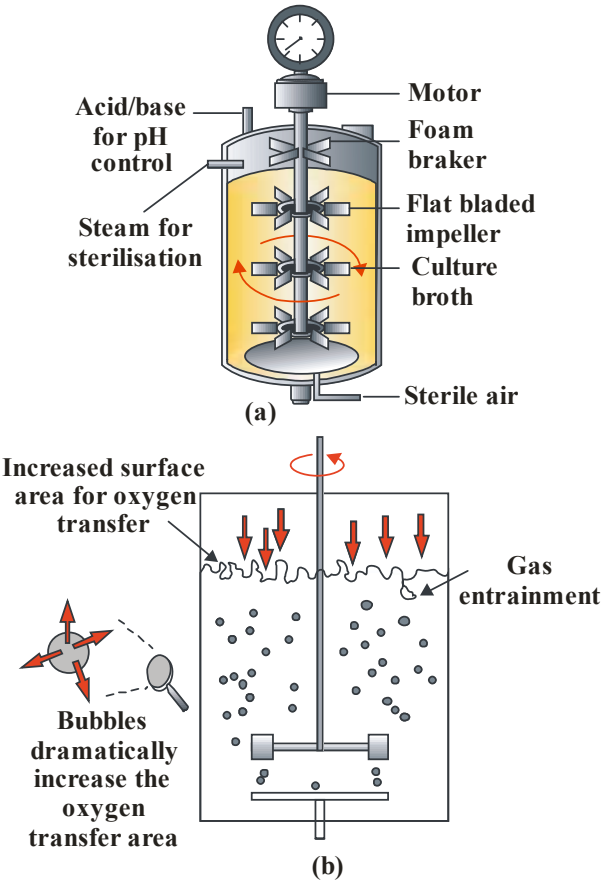


Figure : (a) Simple stirred-tank bioreactor; (b) Sparged stirred-tank bioreactor through which sterile air bubbles are sparged.

Downstream processing:

* After biosynthesis inside the bioreactor, the product has to be subjected through a series of processes before it is ready for marketing.
* The process includes separation and purification, which are collectively referred as downstream processing.
* The product has to be formulated in suitable preservatives.
* Such formulation has to undergo through clinical trials as in case of drugs.

CONCEPT REVIEW

* **Recombinant DNA technology** isolates and amplifies specific sequences of DNA by incorporating them into **vector** DNA molecules. Researchers then propagate and amplify the resulting recombinant DNA in organisms such as *E. coli.*
* Researchers use **restriction enzymes** to cut DNA into specific fragments. Each type of restriction enzyme recognizes and cuts DNA at a highly specific base sequence. Many restriction enzymes cleave DNA sequences to produce complementary, single-stranded sticky ends.
* Geneticists construct the most common recombinant DNA vectors from naturally occurring circular bacteria DNA molecules called **plasmids**, or from bacterial viruses called **bacteriophages**.
* Geneticists often construct recombinant DNA molecules by allowing the ends of a DNA fragment and a plasmid (both cut with the same restriction enzyme) to associate by complementary base pairing. Then **DNA ligase** covalently links the DNA strands to form a stable recombinant molecule.
* A **genomic library** contains thousands of DNA fragments that represent the total DNA of an organism, and a **chromosome library** contains all the DNA fragments from a specific chromosome. Each DNA fragment of a genomic or chromosome library is stored in a specific bacterial strain. Analyzing DNA fragments in genomic and chromosome libraries yields useful information about genes and their encoded proteins.
* A **cDNA library** is produced using **reverse transcriptase** to make DNA copies of mature mRNA isolated from eukaryotic cells. These copies, known as **complementary DNA (cDNA)**, are then incorporated into recombinant DNA vectors.
* Genes present in genomic and chromosome libraries from eukaryotes contain **introns**, regions that do not code for protein. Those genes are amplified in bacteria, but the protein is not properly expressed. Because the introns have been removed from mRNA molecules, eukaryotic genes in cDNA libraries can sometimes be expressed in bacteria, which produce functional protein products.
* Researchers use a radioactive DNA or RNA sequence as a **genetic probe** to identify complementary nucleic acid sequences. Each spot on the x-ray film identifies a colony containing a plasmid that includes the DNA of interest.
* In the **Southern blot technique**, researchers separate DNA fragments by **gel electrophoresis**, denature them, and then blot them onto a nitrocellulose or nylon membrane. A radioactive probe is then **hybridized** by complementary base pairing to the DNA bound to the membrane, and the radioactive band or bands of DNA are identified by autoradiography or chemical luminescence.
* The **polymerase chain reaction (PCR)** is a widely used, automated, in vitro technique in which researchers target a particular DNA sequence by specific primers and then **clone** it by a heat-resistant DNA polymerase.
* **DNA sequencing** yields information about the structure of a gene and the probable amino acid sequence of its encoded proteins. Geneticists compare DNA sequences with other sequences stored in massive databases.
* Automated DNA sequencing is based on the chain termination method, which uses dideoxynucleotides, each tagged with a differently colored fluorescent dye, to terminate elongation

during DNA replication. Gel electrophoresis separates the resulting fragments, and a laser identifies the nucleotide sequence.

* **DNA typing** is the analysis of an individual's DNA. It is based on a variety of **short tandem repeats (STRs)**, molecular markers that are highly **polymorphic** within the human population. DNA typing has applications in law enforcement, issues of disputed parentage, and tracking tainted foods, to name a few.

IMPORTANT POINTS

* Bacterial plasmid contains DNA.
* Restriction endonucleases are synthesised by bacteria for their defence.
* Restriction enzyme EcoRI cleavage DNA at the sequence = GAATTC.
* Nucleic acid is fragmented by nucleases.
* Cosmids is the vector suitable for cloning long DNA fragments.
* pBR 322 is the plasmid.
* DNA is generally methylated at C-base.
* DNA ligases are used to cleave DNA molecules.
* Golden Rice is variety rich in β-Carotene and ferritin.
* Polymerase chain reaction employs primers and DNA polymerase.
* PCR technique was invented by Karry Mullis.
* Gene amplification using primers can be done by polymerase chain reaction.
* Melting of DNA at 70°C is due to break-down of hydrogen bonds.
* Bacteriophage (bacterial virus) has been used to transfer gene for β galactosidase from Escherichia coli to human cells. Lambda phage (λ phage) has been used for transferring lac genes of E. coli into haploid callus of tomato.

Vector type	**Insert size kb**
Plasmid	0.5-8
Bacterophage lamda	9-23
Cosmid	30-45
BAC	50-300
YAC	1000-2500

* "Dolly" sheep was produced by using nuclear transfer technique by Dr. Ian wilmut and his colleagues at Roslin Institute of Scotland in 1997. They used somatic cells from udder (mammary glands) for forming this clone.
* Alec Jeffreys et al (1985) developed the procedure of genetic analysis and forensic medicine, called DNA finger printing.
* **Table : Exonuclease V/s Endonuclease**

S.No.	Exonuclease	Endonuclease
1.	These cut the end regions of the DNA.	These cut at specific regions within the DNA.
2.	These act on single strand of DNA.	These act on both strands as well as on DNA strand.

QUESTION BANK

EXERCISE - 1 (LEVEL-1) [NCERT EXTRACT]

SECTION - 1 (VOCABULARY BUILDER)

Choose one correct response for each question.

For Q.1-Q.5

Match the column I with column II.

Q.1

	Column I	Column II
a.	*Eco* RI	i. *E. coli* R 245
b.	*Hind* III	ii. *Bacillus amyloiquefaiens*
c	*Bam* HI	iii. Haemophilus influenzae
d.	*Eco* RII	iv. *Escherichia coli* RY13

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

Q.2

	Column I	Column II
(a)	Plasmids	(i) Natural polymer of D-galactose
(b)	Bacteriophages	(ii) Hybrid vector derived from plasmids.
(c)	Cosmids	(iii) Virus infecting bacteria
(d)	Agarose	(iv) Circular extra-chromosomal DNA

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

Q.3

	Column I	Column II
a.	PCR	i. Join or hybridise
b.	Denaturation	ii. Polymerisation
c.	Annealing	iii. Melting of target DNA
d.	Extension	iv. Kary Mullis

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

Q.4

	Column I	Column II
(a)	Recombinant DNA	(i) Sea weeds
(b)	Gel electrophoresis	(ii) DNA staining
(c)	Ethidium bromide	(iii) Plasmid DNA that has incorporated human DNA
(d)	Agarose	(iv) Process by which DNA fragments are separated based on their size.

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

Q.5

	Column I	Column II
(a)	Gel electrophoresis technique	i. Har Govind Khorana
(b)	Father of genetic engineering	ii. Dr. Lalji Singh
(c)	Father of Indian DNA fingerprinting	iii. Paul Berg
(d)	DNA ligase in T_4-bacteriophage	iv. A Tiselius

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

SECTION - 2 (BASIC CONCEPTS BUILDER)

For Q.6 to Q.15 :

Choose one word for the given statement from the list.

Calcium, Ethidium bromide, BAC–Bacterial Artificial chromosome vectors, Polymerase chain reaction, Multiple cloning site, *Thermus aquaticus*, Transformation, Magnesium, Elution, Genetic engineering.

Q.6 After separation of nucleic acid molecules by gel electrophoresis, compound used for visualizing the separated molecules is _____.

Q.7 Unique restriction sites are present in a small region of DNA in a vector. This region called _____.

Q.8 The vector based on F plasmid of E. coli is _____.

Q.9 Cations of _____ enable efficient entry of DNA into bacterium through pores in its cell wall.

Q.10 For DNA polymerase to be functional divalent cations of _____ are essential.

Q.11 The diagnostic technique that aids in detection of specific mutations is _____.

Q.12 The method where complete bacterial cells take up DNA from their surroundings after a heat shock is _____.

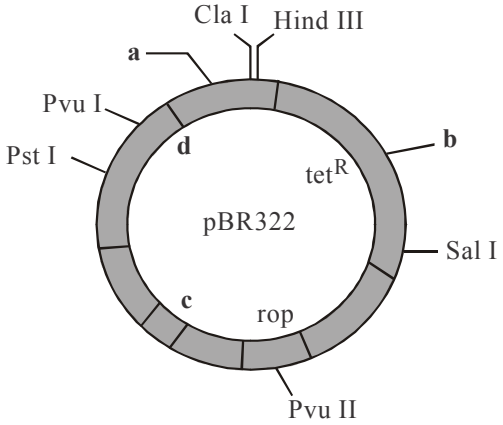
Q.13 The experimental manipulation of DNA of different species producing recombinant DNA is known as _____.

Q.14 In gel electrophoresis, the separated bands of DNA are cut out and extracted from the gel piece This step is called ____.

Q.15 *Taq* polymerase enzyme used in PCR is isolated from _____.

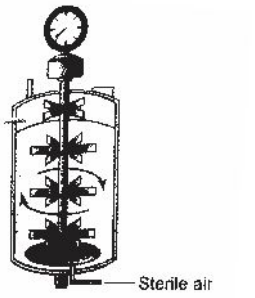
SECTION - 3 (ENHANCE DIAGRAM SKILLS)

Q.16 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

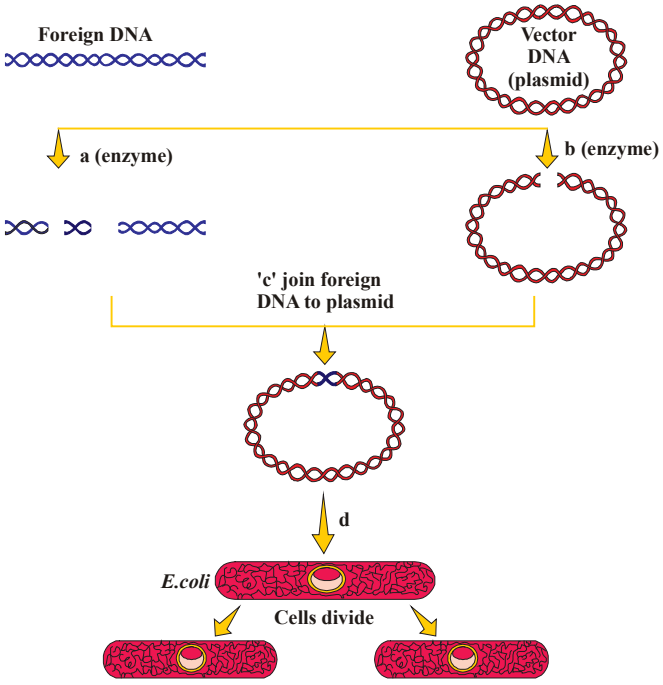
Q.17 Identify the correct match for the given diagram



Apparatus **Function**

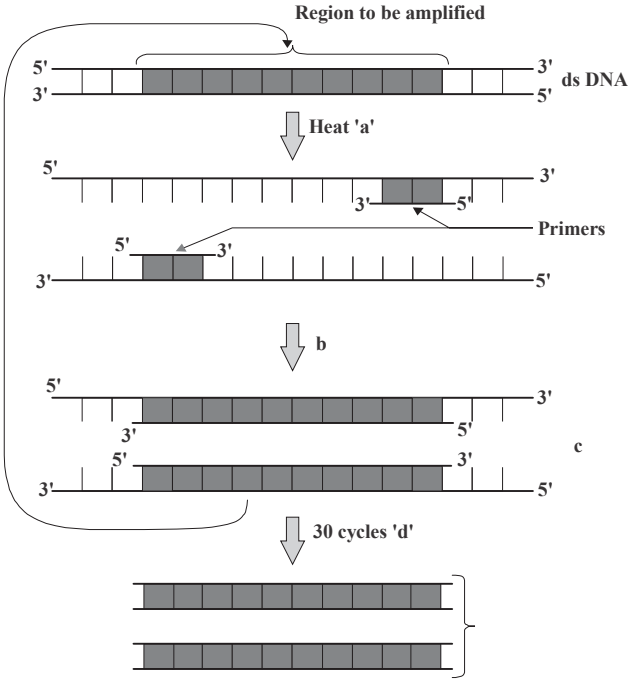
(A) Gene gun – Vectorless direct gene transfer
(B) Electrophoresis – Differential migration of DNA fragments
(C) Bioreactor– Raw materials are biologically converted into specific products
(D) Respirometer – Finding out rate of respiration.

Q.18 The flowchart given below represent the process of recombinant technology. Identify a to d.



(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 ligase, d-Transformation

Q.19 The below diagram refer to PCR. Identify the steps a, b and c and 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-Amplification
(B) A-Annealing at 94-96°C, b-Denaturation at 40-60°C c-Extension through taq polymerase d-Amplification
(C) a-Extension through taq polymerase at 40-60°C, b-Amplificaion, 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

SECTION - 4 (ENHANCE PROBLEM SOLVING SKILLS)

Choose one correct response for each question.

PART - 1 : PRINCIPLES OF BIOTECHNOLOGY

Q.20 The restriction enzyme(s) used in recombinant DNA technology that make staggered leaving sticky ends is/are cuts in

(A) *Eco* RI (B) *Hind* III
(C) *Bam* HI (D) All of these

Q.21 An antibiotic resistance gene in a vector usually helps in the selection of –
(A) competent cells (B) transformed cells
(C) recombinant cells (D) None of these

Q.22 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 living cell
(C) collection of entire genome in form of plasmid
(D) enzyme, cuts the DNA at specific sites

Q.23 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

Q.24 The first recombinant DNA was constructed by
(A) Stanley Cohen
(B) Herbert Boyer
(C) Both (A) and (B)
(D) Temin and Baltimore

Q.25 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

Q.26 Two enzymes responsible for restricting the growth of bacteriophages in *E. coli* were isolated. One was methylase and other was restriction endonuclease. What is the significance of methylase?
(A) Protection of host DNA from the action of restriction endonuclease by adding methyl group to one or two bases usually with in the sequence recognised by restriction enzyme
(B) Able to ligate the two cohesive ends of DNA molecule.
(C) Able to remove the methyl group and hence, prevent the action of restriction endonuclease on host DNA.
(D) Able to cut the DNA of bacteriophage at specific sites.

Q.27 Which of the following methods(s) is used to introduce foreign DNA into host cells?
(A) Gene gun method (B) Gel electrophoresis
(C) Elution (D) Extension

Q.28 Which of the following is a plasmid?
(a) pBR 322 (b) *Bam* II
(C) *Sal* I (D) *Eco* RI

Q.29 The transfer of genetic material from one bacterium to another through the mediation of a vector like virus is termed as:
(A) Transduction (B) Conjugation
(C) Transformation (D) Translation

Q.30 Restriction enzymes are used to cut
(A) single stranded RNA
(B) double stranded DNA
(C) single-stranded DNA
(D) double-stranded RNA

Q.31 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 the above

Q.32 Plants in comparison to animals are more rapidly manipulated by genetic engineering. Select out the most probable reason for this –
(A) totipotency shown by plant cells
(B) single somatic cell can regenerate a whole plant body.
(C) genetic engineering is supplemented with plant tissue culture techniques
(D) All of the above

Q.33 Restriction enzyme was discovered by –
(A) Alexander Flemming
(B) Waksman
(C) Berg
(D) Smith, Nathan and Arber

Q.34 In gel electrophoresis, the sample DNA is fragments by –
(A) restriction endonucleases
(B) exonuclease
(C) endonuclease
(D) anhydro L-galactose

Q.35 Which of the given statement is correct in the context of observing DNA 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.

Q.36 The most important feature in a plasmid to be used as a vector is
(A) origin of replication
(B) presence of a selectable marker
(C) presence of sites for restriction endonuclease
(D) its size

Q.37 Transfer of any gene into a completely different organism can be done through –
(A) genetic engineering (B) tissue culture
(C) transformation (D) None of these

Q.38 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

Q.39 Identify the palindromic sequence in the following

(A) $\frac{GAATTC}{CTTUUG}$ (B) $\frac{GGATCC}{CCTAGG}$

(C) $\frac{CCTGGA}{GGACCT}$ (D) $\frac{CGATAC}{GCTAAG}$

Q.40 An enzyme catalysing the removal of nucleotides from the ends of DNA is –
(A) endonuclease (B) exonuclease
(C) DNA ligase (D) *Hind* II

Q.41 DNA fingerprinting refers to –
(A) molecular analysis of profiles of DNA samples
(B) analysis of DNA samples using imprinting device.
(C) techniques used for molecular analysis of different specimens of DNA.
(D) techniques used for identification of fingerprints of individuals.

Q.42 GAATTC is the recognition site for the restriction endonuclease –
(A) *Eco* RI (B) *Hind* II
(C) *Eco* RII (D) *Bam* HI

Q.43 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 purime and pyrimidine bases
(D) None of the above

Q.44 '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 in bacteria.
(D) All of the above.

Q.45 Plasmid is –
(A) an autonomously replicating circular extrachromosomal DNA.
(B) an autonomously replicating circular extrachromosomal RNA.
(C) an circular protein molecules.
(D) an autonomously replicating chromosomal DNA.

Q.46 Who discovered that restriction enzymes have the capability of cutting DNA strands in a particular fashion, which left what has became known as 'sticky ends' on the strands?
(A) Ramdeo Mishra (B) Stanley Cohen
(C) Herbert Boyer (D) James D Watson

PART - 2 : PROCESSES OF RECOMBINANT DNA TECHNOLOGY

Q.47 Polymerase Chain Reaction (PCR) needs
(A) DNA template (B) primers
(C) *Taq* polymerase (D) All of these

Q.48 Who among the following was awarded the Nobel Prize for the development of PCR technique?
(A) Herbert Boyer (B) Hargovind Khurana
(C) Kary Mullis (D) Arthur Kornberg

Q.49 Source of *taq* polymerase used in PCR is a –
(A) thermophilic fungus
(B) mesophilic fungus
(C) thermophilic bacterium
(D) halophilic bacterium

Q.50 Which of the following techniques can be used to detect genetic disorders in human?
(A) Polymerase Chain Reaction (PCR)
(B) Gel electrophoresis
(C) Spectroscopy
(D) All of the above

Q.51 Which of the following has popularised the PCR (polymerase chain reactions)?
(A) Easy availability of DNA template
(B) Availability of synthetic primers
(C) Availability of cheap deoxyribonucleotides
(D) Availability of 'Thermostable' DNA polymerase

Q.52 A bioreactor is
(A) hybridoma
(B) culture containing radioactive isotopes
(C) culture for synthesis of new chemicals
(D) fermentation tank

Q.53 Which of the following steps are catalysed by *taq* polymerase in a PCR reaction?
(A) Denaturation of template DNA
(B) Anneling of primers to template DNA
(C) Extension of primer end on the template DNA
(D) All of the above

Q.54 While isolating DNA from bacteria, which of the following enzymes is not used?
(A) Lysozyme (B) Ribonuclease
(C) Deoxyribonuclease (D) Protease

Q.55 Thermostable enzymes '*taq*' and '*vent*' isolated from thermophilic bacteria are –
(A) DNA polymerase
(B) DNA ligases
(C) Restriction endonucleases
(D) RNA polymerases

Q.56 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) Any of the above

Q.57 Stirred-tank bioreactors have been designed for
(A) purification of the product.
(B) addition of preservatives to the product.
(C) availability of oxygen throughout the process.
(D) ensuring anaerobic conditions in the culture vessel.

Q.58 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.

Q.59 Gene amplifticaion using primers can be done by
(A) microinjection (B) ELISA
(C) polymerase chain reaction (D) gene gun

Q.60 The polymerase chain reaction is a technique that is used for –
(A) *in vivo* replication of DNA
(B) *in vivo* synthesis of mRNA
(C) *in vitro* synthesis of mRNA
(D) *in vitro* replication of specific DNA sequence using thermostable DNA polymerase.

EXERCISE - 2 (LEVEL-2)

Choose one correct response for each question.

Q.1 cDNA is made by copying –
(A) RNA (B) DNA
(C) proteins (D) plasmids

Q.2 The Ti plasmid, carried by *Agrobacterium tumefaciens*, is especially useful for introducing genes into
(A) bacteria (B) plants
(C) animals (D) yeast

Q.3 A genomic library –
(A) represents all the DNA in a specific chromosome.
(B) is made using reverse transcriptase.
(C) is stored in a collection of recombinant bacteria.
(D) is a DNA copy of mature mRNAs.

Q.4 In PCR, which of the following is used as the primer?
(A) RNA (B) DNA
(C) proteins (D) plasmids

Q.5 Most common matrix is agarose a natural polymer used in gel electrophoresis is extracted from :
(A) an animal (B) a fungus
(C) Sea weeds (D) None of these

Q.6 To isolate DNA from the plant cells we have to break the wall this is done by :
(A) Lysozyme (B) Cellulase
(C) Chitinase (D) Invertase

Q.7 Which of the following enzyme is used in PCR?
(A) RNA polymerase (B) DNA polymerase
(C) reverse transcriptase (D) DNA ligase

Q.8 A plasmid –
(A) is used as a DNA vector
(B) is a type of bacteriophage
(C) is a type of cDNA
(D) is a retrovirus

Q.9 To make cell competent to take up DNA, heat shock is given to cells, the temperature of shock is :
(A) 30°C (B) 42°C
(C) 60°C (D) 90°C

Q.10 Gel electrophoresis is used to separate
(A) DNA (B) RNA
(C) protein (D) Any of the above

Q.11 Which of the most accurately describes sticky ends?
(A) Bacterial plasmids with insulin gene
(B) mRNA isolated from human cells
(C) DNA fragments with single stranded ends
(D) Proteins that stick to DNA

Q.12 Exonuclease removes nucleotides from
(A) Specific positions (B) the ends of the DNA
(C) any where in DNA (D) All the above

Q.13 Which of the following does the enzyme reverse transcriptase use as a template?
(A) RNA (B) DNA
(C) proteins (D) plasmids

Q.14 When cut by the restriction enzyme, the DNA fragments can be joined together using :
(A) DNA polymerase (B) DNA ligase
(C) Alkaline phosphatase (D) DNA gyrase

Q.15 In gel electrophoresis the DNA fragments separate according to size (smaller the fragment size, the faster it moves) this effect is called :
(A) Sieving effect (B) Movement effect
(C) Size effect (D) Spooling

Q.16 Enzyme reverse transcriptase is obtained from.
(A) bacteria (B) virus
(C) humans (D) plants

Q.17 A bacterial cell was transformed with a recombinant DNA 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

Q.18 The PCR technique uses
(A) heat-resistant DNA polymerase
(B) reversetranscriptase
(C) DNA ligase
(D) restriction enzymes

Q.19 A cDNA clone contains
(A) introns (B) exons
(C) anticodons (D) A and B

Q.20 Which of the following statements are correct for the enzyme *taq* polymerase?
I. *Taq* polymerase is thermally unstable.
II. It requires primers for carrying out the process of polymerisation.
III. *Taq* polymerase is isolated from thermophilic bacterium, *Thermus aquaticus*.
Choose the correct option.
(A) I and II (B) I and III
(C) II and III (D) I, II and III

Q.21 A restriction endonucleases which always cut DNA molecules at a particular point by recognising a specific sequence of six base pairs is :
(A) Hind-II (B) Psu I
(C) Hae-III (D) All of these

Q.22 Taq. polymerase is obtaned from:
(A) *Bacillus thuriengiensis*
(B) *Thermus aquaticus*
(C) *Salmonella typhimurium*
(D) *Eischerichia coli*

Q.23 The first letter of the name of Restriction endonuclease came from the
(A) Genus of organism
(B) Species of organism
(C) Family of organism
(D) Class of organism

Q.24 Which of the following is correctly matched –
(A) *Agrobacterium tumefaciens* – Tumour
(B) *Thermus aquaticus* – *Bt* gene
(C) pBR322 – Enzyme
(D) Ligase – Molecular scissors

Q.25 Which of the followingis must in Biotechnology?
(A) Restriction endonuclease + DNA ligase
(B) Restriction exonuclease + DNA polymerase
(C) Alkaline phosphate + DNA Ligase
(D) RNA polymerase + DNA polymerase

Q.26 Autonomously replicating circular extra chromosomal DNA of bacteria is :
(A) Plastid (B) Nucleus
(C) Plasmid (D) None of these

Q.27 The most commonly used bioreactors are of
(A) Simple stirring type
(B) Sparged stirring type
(C) Both (A) and (B)
(D) None of the above

Q.28 Taq polymerase is used in, polymerase chain reaction, because :
(A) It becomes inactive at high temperature.
(B) It makes other enzyme active at high temperature.
(C) It remains active during high temperature.
(D) It is obtained from thermostable virus.

Q.29 The vessels, where large volumes of culture can be processed are :
(A) Bioreactors (B) Biovessels
(C) Biocontainers (D) All of above

Q.30 The first restriction endonucleases enzyme was :
(A) Hind - II (B) EcoRI
(C) Hae - III (D) Bam-I

Q.31 Which of the following enzymes is known as 'genetic glue'?
(A) DNA polymerase (B) Alkaline phosphatase
(C) DNA ligase (D) All of the above

Q.32 The enzymes, which remove nucleotides from the ends of the DNA are :
(A) Exonuclease (B) Endonuclease
(C) Cellulase (D) Hydrolase

Q.33 Small chemically synthesised oligonucleotides that are complementary to the regions of DNA at 3' end used in PCR are :
(A) Primers (B) Dimers
(C) Small strands (D) Large fragments

Q.34 Bombardment of high velocity micro-particles of gold or tungsten coated with DNA on target cells is :
(A) Biolistics (B) Micro-injection
(C) Electroporation (D) Bombing

Q.35 Construction of combinant DNA involves –
(A) Cleaving and rejoining DNA segments with ligase alone.
(B) Cleaving DNA segments with ligase and rejoining them with endonuclease.
(C) Cleaving DNA segment with endonuclease and rejoining them with ligase.
(D) Cleaving and rejoining DNA segments with endonuclease alone.

Q.36 A distinct mechanism that usually involves a short segment of DNA with remarkable capacity to move from one location in a chromosome to another, this is called –
(A) DNA replication (B) DNA transposition
(C) DNA hybridization (D) DNA recombination

Q.37 You have three copies of a particular DNA molecule what technique would you use to make more copies of the molecule?
(A) Gel electrophoresis
(B) Sequencing
(C) PCR
(D) Restriction fragment analysis

Q.38 In gel electrophoresis technique the DNA fragments are forced to move through a medium towards :
(A) Anode (B) Cathode
(C) Both (A) and (B) (D) None of the above

Q.39 First recombinant DNA was made by Stanley Cohen and Herbert Boyer in :
(A) 1968 (B) 1970
(C) 1972 (D) 1974

Q.40 Which type of bioreactor is usually cylindrical or with a curved base to facilitate the mixing of the contents?
(A) Sparged tank bioreactor
(B) Stirred tank bioreactor
(C) Both (A) and (B)
(D) None of the above

Q.41 DNA fingerprinting refers to –
(A) Techniques used for molecular analysis of different specimens of DNA.
(B) Techniques used for identification of fingerprints of individuals.
(C) Molecular analysis of profiles of DNA samples.
(D) Analysis of DNA samples using imprinting devices.

Q.42 Which enzyme is used in PCR technique?
(A) Thermostable DNA polymerase
(B) Thermostable RNA polymerase
(C) Thermostable ligase
(D) Thermostable vector

Q.43 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 produces the same kind of sticky ends in different DNA molecules.

Q.44 In micro injection :
(A) DNA is bombarded on target cells.
(B) DNA is placed through a vector.
(C) DNA is directly injected into the nucleus of animal cell.
(D) None of the above

Q.45 DNA molecules with complementary sticky ends associate –
(A) covalent bonds (B) hydrogen bonds
(C) ionic bonds (D) disulfide bonds

Q.46 Downstream processing is :
(A) Process of separation of DNA fragments
(B) Process of joining the vector and the host DNA.
(C) Process including separation and purification of the product.
(D) Process of transferring DNA.

Q.47 Enzymes responsible for restricting the growth of bacteriophage in *E. coli* were isolated in 1963
(A) DNA Ligases
(B) Alkaline phosphatases
(C) DNA polymerases
(D) Restriction endonuclease

Q.48 The specific DNA sequence in a chromosome which is responsible for initiation of replication is
(A) Cloning region (B) Termination region
(C) Initiation region (D) Origin of replication

Q.49 Restriction endonucleases –
(A) Are used for in uitro DNA synthesis.
(B) Are synthesized by bacteria as part of defense mechanism.
(C) Are present in mammalian cells for degradation of DNA when the cells dies.
(D) Are used in genetic engineering for ligating two DNA molecules.

Q.50 Restriction enzymes belong to a larger class of enzymes
(A) Cellulases (B) Hydrolases
(C) Polymerases (D) Nucleases

Q.51 Vector which is commonly used to transfer foreign gene in a crop plant is :
(A) Plasmids of Salmonella
(B) λ bacteriophage vector
(C) Ti plasmid of *Agrobacterium tumifaciens*
(D) None of the above

Q.52 In gel electrophoresis, separated bands of DNA are cut out from the agarose gel and extracted from the gel pieces, This step is known as :
(A) Blotting (B) Elution
(C) Cloning (D) Tagging

Q.53 If any protein encoding gene is expressed in a heterologous host then protein is known as :
(A) Recombinant gene
(B) Recombinant protein
(C) Selectable marker
(D) Homogenous protein

Q.54 Father of genetic engineering is :
(A) Paul Berg (B) Nathans
(C) Herbert Boyer (D) Stanley Cohen

Q.55 A recombinant DNA molecule can be produced in the absence of the following:
(A) Restriction endonuclease (B) DNA ligase
(C) DNA fragments (D) E.coli

Q.56 Alternative selectable markers developed to diiferentiate non-recombinants from recombinants on the basis of :
(A) Ability of separate them according to size
(B) Ability to produce colour in the presence of a chromogenic substrate
(C) Ability to not produce colour
(D) None of the above

Q.57 To denature the DNA template in PCR it is heated to –
(A) 70°C (B) 54°C
(C) 80°C (D) 94°C

Q.58 If the bacterium does not have any insert, then the presence of chromogenic substrate, it gives :
(A) Red coloured colonies
(B) Colourless colonies
(C) Blue colonies
(D) Green colonies

Q.59 Plasmid are suitable vectors for gene cloning because –
(A) these are small circular DNA molecules, which can integrate with host chromosomal DNA.

(B) these are small circular DNA molecules with their replication origin site
(C) these can shuttle between prokaryotic and eukaryotic cells
(D) these often carry antibiotic resistance genes

Q.60 Roman numbers following the names of restriction endonuclease indicate :
(A) The order in which the enzymes were isolated from that strain of bacteria.
(B) strain of bacteria.
(C) the order in which genus is taken to isolate the enzyme.
(D) none of the above

Q.61 In a chromosome there is a specific DNA sequence which is responsible for initiating replication is :
(A) Ori
(B) Palindromic sequence
(B) Initiation sequence
(D) Promoter sequence

Q.62 In PCR-technology primer is a :
(A) Small chemically synthesized oligonucleotide that are complementary to region of DNA.
(B) Large chemically synthesized oligonucleotide that are identical to region of DNA.
(C) Small segment of RNA
(D) None of these

Q.63 A part from DNA in the bacterial nucleoid, there is a circular extrachromosomal DNA in a bacterial cell called:
(A) Plasmid (B) Mesosomes
(C) Chromosome (D) None of these

Q.64 DNA cannot pass through cell membrane as it is
(A) hydrophilic (B) hydrophobic
(C) Lipophilic (D) All the above

Q.65 In the vector pBR322 there is
(A) One selectable marker.
(B) Two selectable markers.
(C) Three selectable markers.
(D) None of the above.

Q.66 When the isolation of genetic material is done the RNA can be removed by treatment with :
(A) Protease (B) Chitinase
(C) Ribonuclease (D) Deoxyribonuclease

Q.67 Knife of DNA :
(A) DNA - ligase (B) Restriction endonuclease
(C) Exonuclease (D) Peptidase

Q.68 Which scientist obtained interferon through recombinant DNA technology –
(A) Kohler and milstein (B) Charles weismann
(C) Nathans & smith (D) An American firm

Q.69 Restriction endonucleases are used in genetic engineering because :
(A) They can degrade harmful proteins.
(B) They can join DNA fragments.
(C) They can cut DNA at variable site.
(D) They can cut DNA at specific base sequences.

Q.70 cDNA is –
(A) formed by reverse transcriptase
(B) cloned DNA
(C) circular DNA
(D) recombinant DNA

Q.71 Blood Stains are found at the site of a murder. If DNA Profiling technique is to be used for identifying the criminal which of the following is ideal for use?
(A) Serum (B) Erythrocytes
(C) Leucocytes (D) Platelets

Q.72 Which of the following components are used in gel electrophoresis?
I. Ethidium bromide
II. Restriction endonuclease
III. Agarose
IV. UV radiation
Choose the correct option.
(A) I and II (B) I and III
(C) I, II and IV (D) I, II, III and IV

EXERCISE - 3 (LEVEL-3)

Choose one correct response for each question.

Q.1 EcoRI recognises palindromic sequence
(A) $^{5'}$GGGCCC$^{3'}$ / $^{3'}$CCCGGG
(B) $^{5'}$-GAATTC-$^{3'}$ / $^{3'}$-CTTAAG$^{5'}$
(C) ^{5}-AAGCTT$^{3'}$ / ^{3}-TTCGAA-^{5}
(D) None of the above

Q.2 In the Southern blot technique, _____ is/are transferred from a gel to a nitrocellulose or nylon membrane.
(A) protein (B) RNA
(C) DNA (D) bacterial colonies

Q.3 In gel electrophoresis the DNA fragment separate according to their size through sieving effect, which is provided by :
(A) Agarose gel (B) Nylone membrane
(C) Polyethylene glycol (D) Ethidium Bromide

Q.4 pBR322 has two antibiotic resistance genes, they are :
(A) Streptomycin and Ampicillin resistant gene
(B) Chloromycetm and tetracycline resistant gene
(C) Tetracycline and neomycin resistant genes
(D) Ampicillin and tetracyclin resistant genes

Q.5 If a recombinant DNA is inserted within the coding sequence of β-galactosidase enzyme then
(A) Non-recombinants will give blue coloured colonies in presence of chromogenic substrate.
(B) Recombinant will give blue coloured colonies in presence of chromogenic substrate.
(C) Both recombinants and non-recombinants give blue colour.
(D) Non-recombinants do not produce colour due to insertional inactivation.

Q.6 When a recombinant DNA is inserted within the coding sequence of an enzyme β-galatosidase, it results into inactivation of the enzyme gene this is called:
(A) Insert inactivation
(B) Insertional inactivation
(C) Insertional activation
(D) None of the above

Q.7 Which of the following sequences is not palindromic?
(A) 5'-AAGCTT-3' / 3'-TTCGAA-5'
(B) 5'-GATC-3' / 3'-CTAG-5'
(C) 5'-GAATTC-3' / 3'-CTTAAG-5'
(D) 5'-CTAA-3' / 3'-GATT-5'

Q.8 Two enzymes responsible for restricting the growth of bacteriophage in *E.coli* were isolated in 1963, one of these cut DNA, while other :
(A) Add propyl group to DNA
(B) Add ethyl group to DNA
(C) Add methyl group to DNA
(D) None of the above

Q.9 The dideoxynucleotides ddATP, ddTTP, ddGTP, and ddCTP are important in DNA sequencing because they –
(A) cause premature termination of a growing DNA strand.
(B) are used as primers.
(C) cause the DNA fragments that contain them to migrate more slowly through a sequencing gel.
(D) are not affected by high temperatures.

Q.10 Palaeontologists unearthed a human skull during excavation. A small fragement of the scalp tissue was still attached to it. Only little DNA could be extracted from it. If the genes of the ancient need to be analysed, the best way of getting sufficient amount of DNA from this extract is –
(A) by hybridising the DNA with a DNA probe.
(B) by subjecting the DNA to polymerase chain reaction.
(C) by subjecting the DNA to gel electrophoresis
(D) by treating the DNA with restriction enduonucleases.

Q.11 This method of finding a gene is used when researchers know very little about the gene they are trying to find. This process results in a complete gene library : a collection of copies of DNA fragments that represent the entire genome of an Organism. Identify the method.

(A) Cloning (B) Shotgun cloning
(C) Gene synthesis (D) None of these

Q.12 Which of the following pair are correctly matched?

(A) Central dogma – Codon
(B) Okazaki fragments – Splicing
(C) RNA polymerase – DNA primer
(D) Restriction enzyme – Genetic engineering

Q.13 These highly polymorphic molecular markers are useful in DNA typing:

(A) short tandem repeats
(B) cloned DNA sequences
(C) palindromic DNA sequences
(D) cosmid cloning vectors

Q.14 Human DNA and a particular plasmid both have sites that are cut by the restriction enzymes Hind III and EcoRI. To make recombinant DNA, the scientist should –

(A) cut the plasmid with EcoRI and the human DNA with Hind III.
(B) use EcoRI to cut both the plasmid and the human DNA.
(C) use Hind III to cut both the plasmid and the human DNA
(D) B or C

Q.15 Which one is not a basic step in genetically modifying an organism

(A) Identification of DNA with desirable genes
(B) Introduction of the identified DNA into the host.
(C) Introduction of unidentified DNA into the host.
(D) Maintenance of introduced DNA in the host and transfer of the DNA to its progeny.

Note (Q.16-Q.18) :

(A) Statement- 1 is True, Statement-2 is True, Statement-2 is a correct explanation for Statement -1
(B) Statement-1 is True, Statement-2 is True ; Statement-2 is NOT a correct explanation for Statement-1.
(C) Statement - 1 is True, Statement- 2 is False.
(D) Statement -1 is False, Statement -2 is False.

Q.16 **Statement 1 :** The uptake of DNA during transformation is an active, energy requiring process.
Statement 2 : Transformation occurs in only those bacteria, which possess the enzymatic machinery involved in the active uptake and recombination.

Q.17 **Statement 1:** In recombinant DNA technology, human genes are often transferred into bacteria (prokaryotes) or yeast (eukaryote).
Statement 2 : Both bacteria and yeast multiply very fast to form huge population which express the desired gene.

Q.18 **Statement 1:** *Agrobacterium tumefaciens* is popular in genetic engineering because this bacterium is associated with roots of all cereals and pulse crops.
Statement 2 :A gene incorporated in the bacterial chromosomal genome gets automatically transferred to the crop with which the bacterium is associated.

Q.19 Downstream process includes

I. Separation of the product from the reactor.
II. Purification of the product.
III. Formation of the product with suitable preservatives.
IV. Quality control testing and clinical trials in case of drugs.

Which of the statement given above are correct?

(A) I, II and III (B) I, II and IV
(C) II, III and IV (D) I, II, III and IV

EXERCISE - 4 (PREVIOUS YEARS AIPMT/NEET EXAM QUESTIONS)

Choose one correct response for each question.

Q.1 The colonies of recombinant bacteria appear white in contrast to blue colonies of non-recombinant bacteria because of– **[NEET 2013]**
(A) insertional inactivation of alpha galactosidase in recombinant bacteria.
(B) inactivation of glycosidase enzyme in recombinant bacteria
(C) non-recombinant bacteria containing beta galactosidase.
(D) insertional inactivation of alpha galactosidase in non-recombinant bacteria.

Q.2 Which of the following is not correctly matched for the organism and its cell wall degrading enzyme– **[NEET 2013]**
(A) Algae – Methylase
(B) Fungi – Chitinase
(C) Bacteria – Lysozyme
(D) Plant cells – Cellulase

Q.3 DNA fragments generated by the restriction endonucleases in a chemical reaction can be separated by – **[NEET 2013]**
(A) electrophoresis
(B) restriction mapping
(C) centrifugation
(D) polymerase chain reaction

Q.4 An analysis of chromosomal DNA using the Southern hybridization technique does not use **[AIPMT 2014]**
(A) electrophoresis (B) blotting
(C) autoradiography (D) PCR

Q.5 *In vitro* clonal propagation in plants is characterized by – **[AIPMT 2014]**
(A) PCR and RAPD
(B) northern blotting
(C) electrophoresis and HPLC
(D) microscopy

Q.6 Which vector can clone only a small fragment of DNA? **[AIPMT 2014]**
(A) Bacterial artificial chromosome.
(B) Yeast artificial chromosome
(C) Plasmid
(D) Cosmid

Q.7 Commonly used vectors for human genome sequencing are **[AIPMT 2014]**
(A) T - DNA (B) BAC and YAC
(C) expression vectors (D) T/A cloning vectors

Q.8 The DNA molecule to which the gene of interest is integrated for cloning is called – **[RE-AIPMT 2015]**
(A) template (B) carrier
(C) transformer (D) vector

Q.9 The cutting of DNA at specific locations became possible with the discovery of **[RE-AIPMT 2015]**
(A) selectable markers (B) ligases
(C) restriction enzymes (D) probes

Q.10 Which of the following is not a feature of the plasmids? **[NEET 2016 PHASE 1]**
(A) Independent replication (B) Circular structure
(C) Transferable (D) Single-stranded

Q.11 The taq polymerase enzyme is obtained from **[NEET 2016 PHASE 1]**
(A) *Thermus aquaticus*
(B) *Thiobacillus ferroxidans*
(C) *Bacillus subtilis*
(D) *Pseudomonas putida*

Q.12 Which of the following is a restriction endonuclease? **[NEET 2016 PHASE 1]**
(A) Hind II (B) Protease
(C) DNase I (D) RNase

Q.13 Stirred-tank bioreactors have been designed for **[NEET 2016 PHASE 2]**
(A) Purification of product.
(B) Addition of preservatives to the product.
(C) Availability of oxygen throughout the process.
(D) Ensuring anaerobic conditions in the culture vessel.

Q.14 A foreign DNA and plasmid cut by the same restriction endonuclease can be joined to form a recombinant plasmid using **[NEET 2016 PHASE 2]**

(A) Eco RI (B) Taq polymerase
(C) Polymerase III (D) Ligase

Q.15 Which of the following is not a component of downstream processing? **[NEET 2016 PHASE 2]**

(A) Separation (B) Purification
(C) Preservation (D) Expression

Q.16 Which of the following restriction enzymes produces blunt ends? **[NEET 2016 PHASE 2]**

(A) Sal I (B) Eco RV
(C) Xho I (D) Hind III

Q.17 A gene whose expression helps to identify transformed cell is known as – **[NEET 2017]**

(A) Selectable marker (B) Vector
(C) Plasmid (D) Structural gene

Q.18 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

Q.19 The DNA fragments separated on an agarose gel can be visualised after staining with : **[NEET 2017]**

(A) Bromophenol blue (B) Acetocarmine
(C) Aniline blue (D) Ethidium bromide

Q.20 What is the criterion for DNA fragments movement on agarose gel during gel electrophoresis? **[NEET 2017]**

(A) The larger the fragment size, the farther it moves.
(B) The smaller the fragment size, the farther it moves.
(C) Positively charged fragments move to farther end.
(D) Negatively charged fragments do not move.

Q.21 DNA fragments are **[NEET 2017]**

(A) Positively charged
(B) Negatively charged
(C) Neutral
(D) Either positively or negatively charged depending on their size.

Q.22 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

Q.23 Which one of the following equipments is essentially required for growing microbes on a large scale, for industrial production of enzymes? **[NEET 2019]**

(A) BOD incubator (B) Sludge digester
(C) Industrial oven (D) Bioreactor

Q.24 DNA precipitation out of a mixture of biomolecules can be achieved by treatment with **[NEET 2019]**

(A) Isopropanol
(B) Chilled ethanol
(C) Methanol at room temperature
(D) Chilled chloroform

Q.25 Following statements describe the characteristics of the enzyme Restriction Endonuclease. Identify the **INCORRECT** statement. **[NEET 2019]**

(A) The enzyme cuts DNA molecule at identified position within the DNA.
(B) The enzyme binds DNA at specific sites and cuts only one of the two strands.
(C) The enzyme cuts the sugar-phosphate backbone at specific sites on each strand.
(D) The enzyme recognizes a specific palindromic nucleotide sequence in the DNA.

ANSWER KEY

EXERCISE-1 (SECTION-1&2)

(1) (C) **(2)** (B) **(3)** (D)
(4) (A) **(5)** (B) **(6)** Ethidium bromide
(7) Multiple cloning site.
(8) BAC–Bacterial Artificial chromosome vectors
(9) Calcium **(10)** Magnesium
(11) Polymerase chain reaction **(12)** Transformation
(13) Genetic engineering **(14)** Elution
(15) *Thermus aquaticus*

EXERCISE - 1 [SECTION-3 & 4]

Q	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
A	A	C	B	A	D	B	B	C	C	A	A	A	A	A	B	B	D	D	A	D	A	A	C	B	B
Q	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60					
A	A	A	A	C	A	C	D	C	C	A	D	D	C	C	A	C	C	C	C	D					

EXERCISE - 2

Q	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A	A	B	C	B	C	B	B	A	B	D	C	B	A	B	A	B	A	A	B	C
Q	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
A	A	B	A	A	A	C	C	C	A	A	C	A	A	A	C	B	C	A	C	B
Q	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
A	C	A	C	C	B	C	D	D	B	D	C	B	B	A	D	B	D	C	B	A
Q	61	62	63	64	65	66	67	68	69	70	71	72								
A	A	A	A	A	B	C	B	B	D	A	C	D								

EXERCISE - 3

Q	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
A	B	C	A	D	A	B	D	C	A	B	B	D	A	D	C	A	A	D	D

EXERCISE - 4

Q	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
A	C	A	A	D	A	C	B	D	C	D	A	A	C	D	D	B	A	B	D	B	B	D	D	B	B

SOLUTIONS

EXERCISE-1

(1) (C) **(2)** (B) **(3)** (D)
(4) (A) **(5)** (B) **(6)** Ethidium bromide
(7) Multiple cloning site.
(8) BAC–Bacterial Artificial chromosome vectors
(9) Calcium **(10)** Magnesium
(11) Polymerase chain reaction **(12)** Transformation
(13) Genetic engineering **(14)** Elution
(15) *Thermus aquaticus* **(16)** (A)
(17) (C) **(18)** (B) **(19)** (A) **(20)** (D)
(21) **(B).**
(22) **(B).** Vector is a plasmid or virus DNA used to introduce genes into a host cell, where the genes may be amplified (gene cloning) or otherwise manipulated.
(23) **(C).**
(24) **(C).** Stanley Cohen and Herbert Boyer generated first recombinant DNA molecule by combining a gene from a bacterium with plasmid of *Escherichia coil*.
(25) **(A).** Recombinant DNA molecule is a vector (e.g., Plasmid, phage or Virus) into which the desired DNA fragment has been inserted to enable its cloning in an appropriate host. pBR322 of *E. coli* was the first most widely used plasmid for construction of recombinant DNA.
(26) **(A).**
(27) **(A).** Gene gun method was first developed by Prof. Stanford and coworkers at Cornell University, USA in 1987. This method is used to introduce foreign DNA into host cell.
(28) **(A).** Plasmid is an extrachromosomal genetic elements of DNA or RNA that is capable of replicating independetly of the host chromosome e.g. *E. coli* plasmid pBR322.
(29) (A)
(30) **(B).** Restriction enzymes cut double stranded DNA molecules at specific sites called recognition site that have specific base sequence. The restriction enzyme Eco RI, Bam II and Hind III are used in recombinant DNA technology to produce cuts in vector and other DNA molecules to obtain chimeric DNA.
(31) **(B).** Migration rate of DNA molecule in agarose gel electrophoresis depends on its size and conformation
(32) **(D).**
(33) **(D).** Restriction enzyme are known as molecular knives or molecular scissors and are used to cut DNA at specific sites of DNA. These were first discovered by Smith, Nathan and Arber.
(34) (A) **(35)** (D) **(36)** (A) **(37)** (A)
(38) **(C).** Different kinds of specific enzymes are used in genetic engineering, e.g., cleaving enzymes – These enzymes are used to break DNA molecules. They are of three types : (i) Exonucleases (ii) Endonucleases (iii) Restriction endonucleases.
(39) **(B).** Special sequence in the DNA recognised by restriction endonuclease is called palindromic nucleotide sequence.
Restriction endonuclease recognises palindromic sequences in DNA and cuts them.
The palindromes in DNA are base pair sequences that are the same when read forward (left to right) or backward (right to left) from a central axis of symmetry.
For example,
(i) 5' – G A A T T C – 3'
3' – C T T A A G – 5'
(ii) 5' – G G A T C C – 3'
3' – C C T A G G – 5'
(40) **(B).** Exonuclease remove nucleotides from the terminal ends (either 5' or 3') of DNA in one strand of duplex.
(41) **(A).** Important for DNA fingerprinting are short nucleotide repeats that very in number from person to person but are inherited. These are Variable Number Tandem Repeats or VNTRs and these can be recognised only through molecular analysis of DNA samples. Alec Jeffreys (1985, 86) discovered this technique for the first time.

(42) **(A).** The restriction endonuclease Eco RI is obtained from *Escherichia coli* RY1 3. The recognition sequence for this is GAATTC, CTTAAG.

(43) **(A).** DNA Ligases (joining or sealing enzymes) are also called genetic gum. They join two individual fragments of double stranded DNA by forming phosphodiester bonds between them. Thus, they help in sealing gaps in DNA fragments. Therefore, they act as a molecular glue. The enzyme used most often in the rDNA technology is T4 DNA ligase.

(44) **(C).** A restriction enzyme is an enzyme that cuts DNA at or near a specific recognition nucleotide sequences known as restriction sites. These enzymes are found in bacteria and provide a defense mechanism against invading virus. Inside a prokaryote, the restriction enzyme cuts up foreign DNA in a process called 'restriction' while host DNA is protected by a modification enzyme that modifies te prokaryotic DNA and blocks cleavage. Together, these processes form restriction modification system.

(45) (A) **(46)** (C)

(47) **(D).** PCR is a technique of synthesising multiple copies of the desired gene or (DNA) *in Vitro*. The basic requirement of PCR are DNA template, two nucleotide primers and enzyme (DNA polymerase).

(48) (C) **(49)** (C)

(50) **(A).** By using PCR phenylketonuria, muscular dystrophy, sickle-cell anaemia, hepatitis chiamydia and tuberculosis can be diagnosed.

(51) **(D).**

(52) **(D).** Bioreactors (fermenters) are considered as vessel in which raw material are biologically converted into specific products by microbes, plant and animal cells and/or their enzymes.

(53) **(C).** During extension, the enzymes Taq polymerase synthesises the DNA segment between the primers. The two primers extend towards each other in order to copy the DNA segment typing between the two primers. This step requires presence of deoxynucleoside triphosphate (d NTPs) and Mg^{2+} and occurs at 72°C.

(54) **(C).**

(55) **(A).** Thermostable enzymes 'Taq and Vent' isolated from thermophilic bacteria are DNA polymerase.
Taq polymerase, isolated from a *Thermophilic bacterium*, *Thermus aquaticus* and *vent polymerase*, isolated from a thermophilic bacterium *Thermococcus litoralis*.

(56) **(C).** The cells having cloned genes of interest can be grown on a small scale in the laboratory. The cultures may be used for extracting and purifying the desired protein. The cells can also be multiplied in a continuous culture system where the used medium is passed out from one side and fresh medium is added from the other side to maintain the cells in their physiologically most active log/ exponential phase - rapid multiplication of the cells. This type of culturing method produces a larger biomass to get higher yields of desired protein.

(57) **(C).** Each bioreactor has a cylindrical stirred-tank to facilitate the mixing of contents. The stirrer provides facility of mixing the contents as well as the availability of oxygen throughout the process.

(58) **(C).** Transformation is a process by which a cell takes up naked DNA fragment from the environment, incorporates it into its own chromosomal DNA and finally expresses the trait controlled by the incoming DNA. Since DNA is a hydrophilic molecule, it can not pass through membranes, so the bacterial cells must be made competent to take up DNA. This is done by treating them with a specific concentration of a divalent cation, such as calcium (Ca^{2+}) which increases the efficiency with which DNA enters the bacterium through pores in its cell wall. Recombinant DNA (rDNA) can then be forced into such cells by incubating the cell with recombinant DNA on ice, followed by placing them briefly at 42°C (heat

shock), and then putting them back on ice. This enables the bacteria to take up the recombinant DNA.

(59) **(C).** Gene amplification using primers can be done by Polymerase Chain Reaction (PCR). In this reaction, multiple copies of the gene of interest is synthesised in *vitro* using two sets of primers and the enzyme DNA polymerase.
Primers are small chemically synthesised that are complementary to the region of DNA.

(60) **(D).** The polymerase chain reaction is a technique that is used for in vitro replication of specific DNA sequence using thermostable DNA polymerase. The polymerase chain reaction or PCR, was originally invented by Kary Mullis in 1985. Kary Mullis shared the Nobel Prize with Michael Smith in chemistry in 1993.

EXERCISE-2

(1) **(A).** cDNA is made as a complementary copy of mRNA.

(2) (B) **(3)** (C)

(4) **(B).** During the PCR procedure, small DNA sequences are used as the primer to start copying DNA to make multiple copies.

(5) (C) **(6)** (B)

(7) **(B).** DNA polymerase is used to make multiple copies of DNA.

(8) (A) **(9)** (B)

(10) **(D).** Gel electrophoresis can be used to separate DNA, RNA or proteins.

(11) **(C).** When special restriction enzymes are used, DNA is cut to leave short, single stranded ends of the fragments.

(12) (B) **(13)** (A) **(14)** (B) **(15)** (A)

(16) **(B).** Reverse transcriptase is isolated from retroviruses.

(17) **(A).** Eukaryotic genes do not function properly when transferred into bacterial cell because introns are present in eukaryotic cells but are absent in prokaryotic cells. Hence, when bacterial cell is transformed with recombinant DNA is generated using human gene, it could not process it. As a result, no desired protein will be produced.

(18) (A) **(19)** (B)

(20) **(C).** The final step in PCR is extension (polymerisation), where in Taq DNA polymerase synthesises the DNA region between the primers using deoxynucleoside triphosphates and Mg^{2+}. It means the primers are extended towards each other so that the DNA segment lying between the two primer is copied. The optimum temperature for this polymerisation step is 72°C.
Taq polymerase is thermostable enzyme isolated from *Thermophilic bacterium*, *Thermus aquaticus*.

(21) (A)

(22) **(B).** Thermus aquaticus is a thermophilic gram-negative bacterium that has played a key role in the modern revolution in genetic research, genetic engineering, and biotechnology.The use of DNA polymerases from T. aquaticus and other thermophiles in PCR and related applications, such as DNA sequencing, has revolutionized biotechnology.

(23) (A)

(24) **(A).** *Agrobacterium tumefaciens* (updated scientific name *Rhizobium radiobacter*) is the causal agent of crown gall disease (the formation of tumour) in over 140 species of dicot. It is a rod-shapd, Gram negative, soil bacterium (Smith, et. al 1907). Symptoms are caused by the insertion of a small segment of DNA known as T-DNA (transfer DNA) into the plant cell, which is incorporated at a semi-random location into the plant genome.

(25) (A)

(26) **(C).** A plasmid is a small, circular, double-stranded DNA molecule that is distinct from a cell's chromosomal DNA. Plasmids naturally exist in bacterial cells, and they also occur in some eukaryotes. Often, the genes carried in plasmids provide bacteria with genetic advantages, such as antibiotic resistance.

(27) **(C).** A bioreactor is a vessel in which a chemical process is carried out which involves organisms or biochemically active substances derived from such organisms. This process can either be aerobic or anaerobic.

(28) (C) **(29)** (A)

(30) **(A).** HindII is a type II restriction enzyme found in Haemophilus influenzae.

(31) (C)

(32) **(A).** Exonucleases are enzymes that work by cleaving nucleotides one at a time from the end (exo) of a polynucleotide chain.

(33) **(A).** A primer is a strand of short nucleic acid sequences (generally about 10 base pairs) that serves as a starting point for DNA synthesis. It is required for DNA replication because the enzymes that catalyze this process, DNA polymerases, can only add new nucleotides to an existing strand of DNA.

(34) **(A).** A gene gun or a biolistic particle delivery system, originally designed for plant transformation, is a device for injecting cells with genetic information; the inserted genetic material are termed transgenes.

(35) (C) **(36)** (B) **(37)** (C)

(38) **(A).** Gel electrophoresis is a laboratory method used to separate mixtures of DNA, RNA, or proteins according to molecular size.
In gel electrophoresis, the molecules to be separated are pushed by an electrical field through a gel that contains small pores.

(39) (C)

(40) **(B).** A batch stirred tank reactor is the simplest type of reactor. It is composed of a reactor and a mixer such as a stirrer, a turbine wing or a propeller.This reactor is useful for substrate solutions of high viscosity and for immobilized enzymes with relatively low activity. However, a problem that arises is that an immobilized enzyme tends to decompose upon physical stirring. The batch system is generally suitable for the production of rather small amounts of chemicals.

(41) (C) **(42)** (A)

(43) **(C).** More than 900 restriction enzymes have been isolated from over 230 strains of bacteria each of which recognise different recognitions sequences. No restriction enzymes is isolated from viruses.

(44) (C) **(45)** (B)

(46) **(C).** Downstream processing refers to the recovery and purification of biosynthetic products, particularly pharmaceuticals, from natural sources such as animal or plant tissue or fermentation broth, including the recycling of salvageable components and the proper treatment and disposal of waste.

(47) (D) **(48)** (D)

(49) **(B).** Restriction enzymes are DNA-cutting enzymes found in bacteria (and harvested from them for use). Because they cut within the molecule, they are often called restriction endonucleases. In order to be able to sequence DNA, it is first necessary to cut it into smaller fragments.

(50) (D) **(51)** (C)

(52) **(B).** The basic principle behind DNA recovery from agarose gel involves a sequence of bind, wash, and elute steps. Once the gel is in solubilizing buffer, it is applied onto a "spin column," which, upon centrifugation, allows DNA molecules to selectively bind to a silica-filter while the impurities flow through into a collection tube.

(53) **(B).** Recombinant protein is a protein that whose code is carried through a recombinant DNA. The term recombinant DNA implies that two segments of DNA in a plasmid.

(54) (A) **(55)** (D) **(56)** (B) **(57)** (D)

(58) (C)

(59) **(B).** Plasmid is an extrachromodomal genetic material of DNA that is capable of replicating independently of host chromosome. It forms the basis of many cloning vectors used in genetic engineering.

(60) (A) **(61)** (A) **(62)** (A) **(63)** (A)

(64) (A) **(65)** (B) **(66)** (C) **(67)** (B)

(68) (B) **(69)** (D)

(70) **(A).** cDNA (complementary DNA) created from a mature mRNA from a eukaryotic cell with the use of an enzyme known as reverse transcriptase.

(71) **(C).** DNA fingerprinting is a technique to identify a person on the basis of person's DNA specificity. The technique is based on upon the fact that the DNA constitution of an individual carries some specific sequence of nucleotides, which do not carry any informantion for protein synthesis.
Leucocytes are to be used for identifying the criminal because they are nucleated, whereas erythrocytes are enucleated.

(72) **(D).**

EXERCISE-3

(1) (B)

(2) **(C).** A Southern blot is a method used in molecular biology for detection of a specific DNA sequence in DNA samples. Southern blotting combines transfer of electrophoresis -separated DNA fragments to a filter membrane and subsequent fragment detection by probe hybridization.

(3) **(A).** Gel electrophoresis is the standard lab procedure for separating DNA by size (e.g. length in base pairs) for visualization and purification. Electrophoresis uses an electrical field to move the negatively charged DNA toward a positive electrode through an agarose gel matrix.

(4) **(D).** pBR322 DNA is a commonly used plasmid cloning vector in E. coli The molecule is a double-stranded circle. pBR322 contains the genes for resistance to ampicillin and tetracycline, and can be amplified with chloramphenicol.

(5) (A) **(6)** (B) **(7)** (D) **(8)** (C)

(9) (A)

(10) **(B).** PCR is a technique by which small samples DNA can be quickly amplified. Starting with only one gene sized pieces of DNA, this technique is used to make literally billions of copies in only a few hours.

(11) **(B).** Shotgun cloning involves cutting the DNA of the entire genome into pieces with restriction enzyme, inserting these pieces or fragments into bacteria or yeast with plasmids or viruses and allowing the organism to reproduce making copies or clones of the DNA fragments.

(12) **(D).** Restriction enzyme is used in genetic engineering. Restriction enzyme is an endonuclease that recognises a specific DNA base sequence and cleaves both the strands of a DNA at or near that site.

(13) **(A).** A short tandem repeat is a microsatellite, consisting of a unit of two to thirteen nucleotides repeated hundreds of times in a row on the DNA strand.
Most of our DNA is identical to DNA of others. However, there are inherited regions of our DNA that can vary from person to person. Variations in DNA sequence between individuals are termed "polymorphisms". Sequences with the highest degree of polymorphism are very useful for DNA analysis in forensics cases and paternity testing. This activity is based on analyzing the inheritance of a class of DNA polymorphisms known as "Short Tandem Repeats", or simply STRs.

(14) (D) **(15)** (C)

(16) **(A).** Transformation does not involve passive entry of DNA molecules through permeable cell walls and membranes. It does not occur 'naturally' in all species of bacteria, only in those species possessing the enzymatic machinery involved in the active uptake and recombination processes.

(17) **(A).** Bacteria and yeast are easily grow in culture medium and multiply very fast so it is best for making the many copies of recombinant DNA, and express character of desired gene.

(18) **(D).** *Agrobacterium tumefaciens* infects certain plants, in which T_1-plasmid (not chromosomal genome) causes the formation of tumour like growth called a crown gall. *Agrobacterium* does not infect grasses (i.e., cereals.)

(19) **(D).** After the formation of the product in the bioreactors, it undergoes through some processes before a finished product to be

ready for marketing. The processes include (i) separation and (ii) purification of product which are collectively called the downstream processing.

EXERCISE-4

(1) **(C).** The colonies of recombinant bacteria appear white in contrast to blue colonies of non-recombinant bacteria due to inactivation of glycosidase enzyme in recombinant bacteria.

(2) **(A).** In algae, cell wall is made up of cellulose degrades by cellulase.

(3) **(A).** DNA fragments generated by restriction endonucleases in a chemical reaction can be separated by gel electrophoresis.

(4) **(D).** This is a technique of detecting DNA by using a DNA probe. In this technique DNA is separated by gel electrophoresis and then transferred from the gel to membrane by blotting. The DNA was detected from membrane with a DNA probe to complementary bind to DNA the probe was labelled by radio active 32 p the labeled probes hybridise target DNA present in blot this probe is detect by auto radiography. So PCR is not included in it.

(5) **(A).** PCR & RAPD (Random Amplification of Polymorphic DNA) technique are used for the characterisation of in vitro clonal propagation in plants.

(6) **(C).** 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

(7) **(B).** Commonly used vectors for human genome sequencing are BAC (Bacterial artificial chromosome) and YAC (Yeast Artificial chromosome).

(8) **(D).** The DNA molecule to which the gene of interest is integrated for cloning is called vector.

(9) **(C).** Restriction endonucleases enzyme cut the DNA at specific site.

(10) **(D).** Plasmid is extra chromosomal, double stranded circular DNA.

(11) **(A).** Taq polymerase is thermostable DNA polymerase obtained from *Thermus aquaticus*.

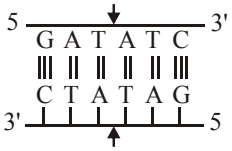
(12) **(A).** Hind II is a restriction endonuclease.

(13) **(C).** Stirred-tank bioreactor is provided with stirrer for availability of oxygen throughout the process.

(14) **(D).** In DNA recombinant technology, linking of foreign DNA and plasmid is made possible by DNA ligase which is also called "molecular glue".

(15) **(D).** After the completion of biosynthetic pathway, downstreaming processing includes all the steps involved in isolation, purification and preservation of products.

(16) **(B).** Eco RV cuts the DNA in the centre of restriction sites forming blunt ends. The pallindromic sequence for Eco RV is



(17) **(A).** In recombinant DNA technology, selectable markers helps in identifying and eliminating non transformants and selectively permitting the growth of the transformants.

(18) **(B).** Biosynthetic stage for synthesis of product in recombinant DNA technology is called upstreaming process while after completion of biosynthetic stage, the product has to be subjected through a series of processes which include separation and purification are collectively referred to as downstreaming processing.

(19) **(D).** Ethidium bromide is used to stain the DNA fragments and will appear as orange coloured bands under UV light.

(20) **(B).** During gel electrophoresis, DNA fragments separate (resolve) according to their size through sieving effect provided by agarose gel.

(21) **(B).** DNA fragments are negatively charged because of phosphate group.

(22) **(D).** This technique is used for making multiple copies of gene (or DNA) of interest in vitro. Each cycle has three steps: (i) Denaturation, (ii) Primer annealing (iii) Extension of primer.

(23) **(D).** To produce enzyme in large quantity equipment required are bioreactors. Large scale production involves use of bioreactors.

(24) **(B).** During the isolation of desired gene, chilled ethanol is used for the precipitation of DNA.

(25) **(B).** Restriction enzymes cut DNA molecules at a particular point by recognising a specific sequence. Each restriction endonuclease functions by inspecting the length of a DNA sequence. Once it 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 sugarphosphate backbone.