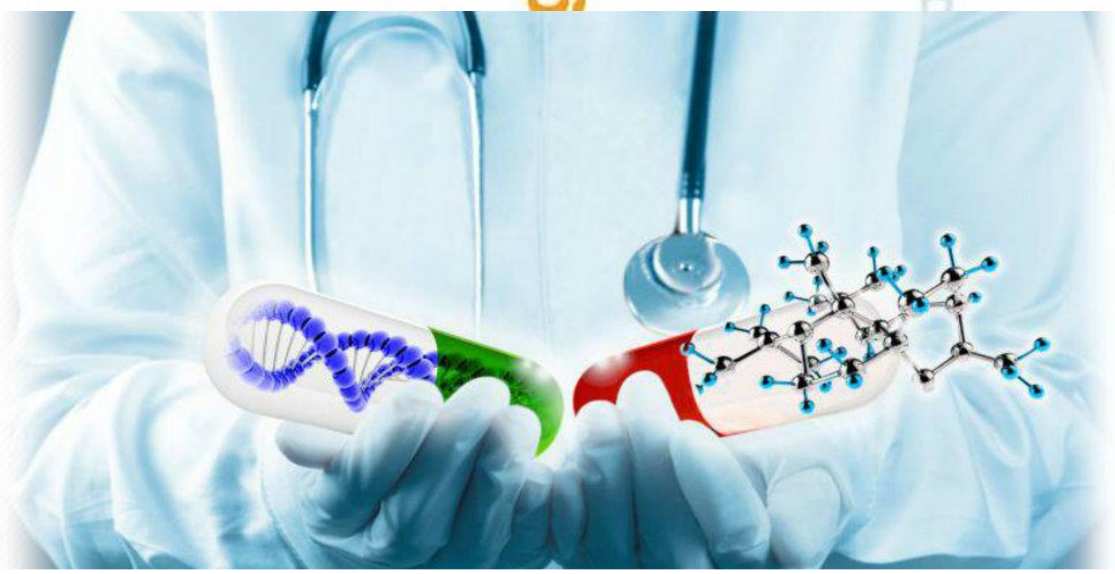
CBSE-XII
BIOLOGY
BIOTECHONOLOGY

BIOTECHNOLOGY - BIOLOGY - CBSE - XII



The Success Destination...

BIOLOGY
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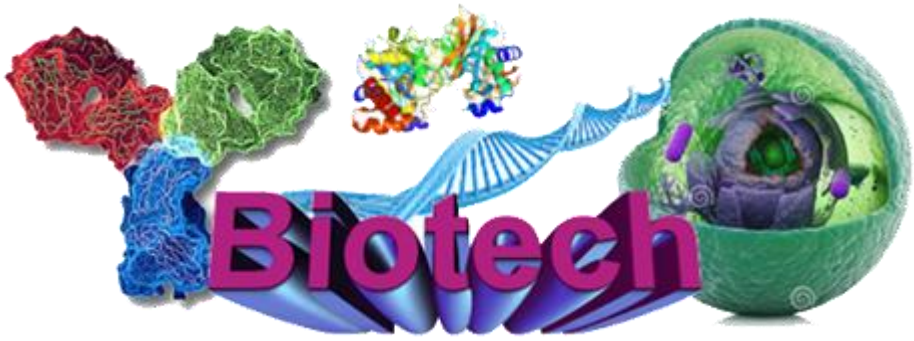


PRINCIPLES PROCESSES

- The term **biotechnology** is derived from the fusion of **biology** and **technology.** Biotechnology means any technological application that uses biological systems, living organisms or their derivatives to produce or modify products or processes for specific use.
- The definition of biotechnology given by the **European Federation of Biotechnology (EFB)** which covers both traditional views and modern molecular biotechnology is as follows: "Biotechnology is the integrated use of biochemistry, microbiology and engineering sciences in order to achieve technological application of the capabilities of microorganisms, cultured tissues/cells and parts there of."

PRINCIPLES OF MODERN BIOTECHNOLOGY

- The science of biotechnology is mainly based on two core technologies :

Techniques of modern biotechnology

Genetic Engineering

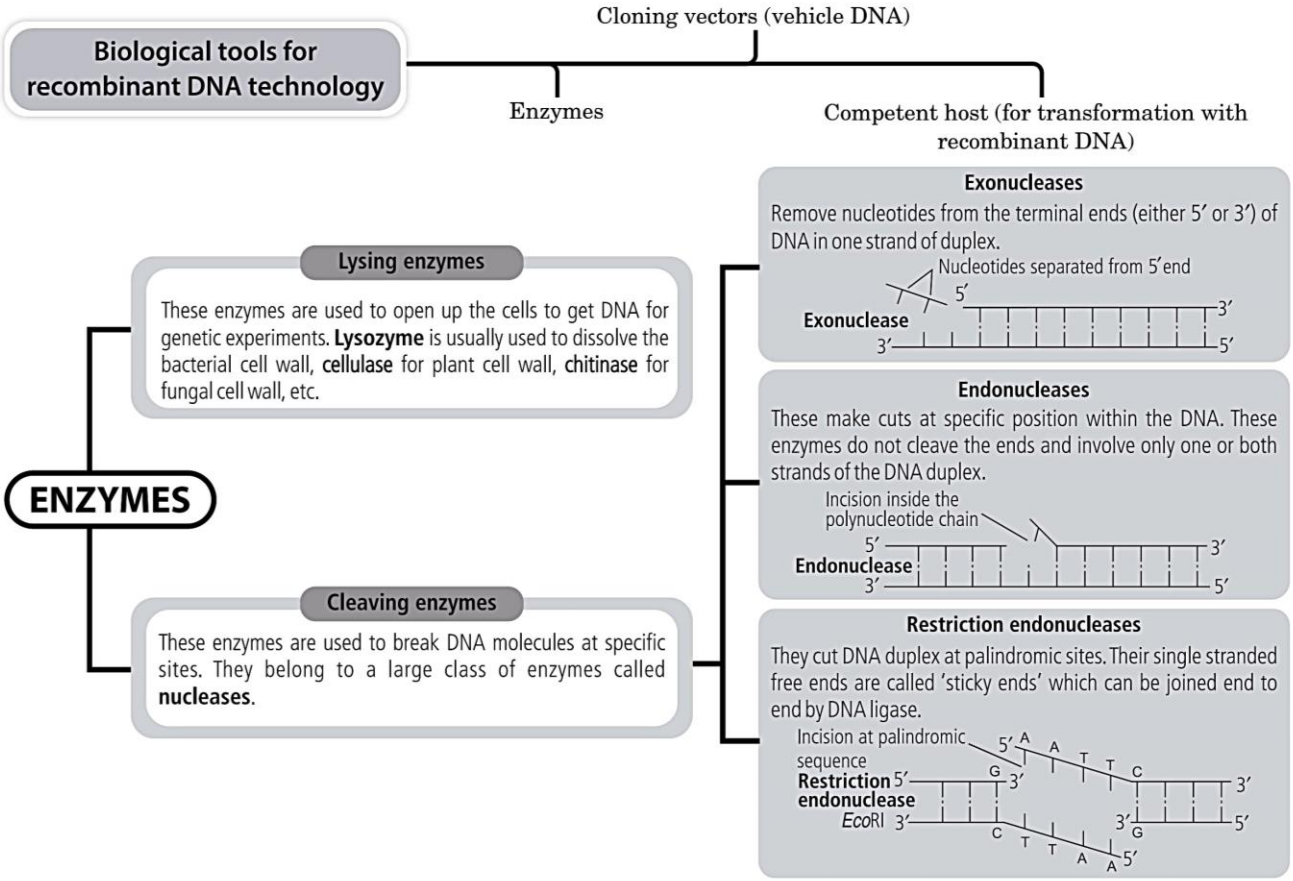
It includes techniques to alter the nature of genetic material of host organism by introducing foreign or recombinant DNA into the host organism thereby changing their phenotype.

Chemical Engineering

It involves maintenance of sterile, microbial contamination free conditions, that allow growth of only the desired microorganism/eukaryotic cell in large quantities, for the manufacture of biotechnological products such as antibiotics, vaccines, enzymes, medicines, hormones, etc.

GENETIC ENGINEERING

- Genetic engineering is the technology involved in synthesis of artificial genes, repair of genes through fusion, deletion, inversion, shifting of genes, products of recombinant DNA & manipulating them for improvement in human beings, plants, animals and microbes.
- An important aspect of genetic engineering is recombinant DNA technology. Recombinant DNA technology is employed for combining DNA from two different organisms to produce recombinant DNA.
- Three basic steps in creating genetically modified organism (GMO) or transgenic organism are:
  (i) Identification of DNA with desirable genes.
  (ii) Introduction of the identified DNA into the host.
  (iii) Maintenance of introduced DNA in the host and the transfer of DNA to its progeny.



RESTRICTION ENDONUCLEASES - THE MOLECULAR SCISSORS

- Restriction endonuclease was isolated for the first time by **W. Arber** in 1962 in bacteria. In 1978, Arber, Smith and Nathan were awarded the Nobel Prize for discovery of restriction endonucleases. These enzymes recognise the base sequence at palindrome sites in DNA duplex and cut its strand.
- The palindromes in DNA are base pair sequences that shows the same sequence on both the strands when read forward (left to right) or backward (right to left) from a central axis of symmetry.
- For example, restriction endonuclease *Eco*RI found in the colon bacteria *E. coli*, recognises the base sequence GAATTC in DNA duplex and cuts the DNA strands between G and A as shown here:

Types of Restriction Endonuclease

- Three main types of restriction endonucleases are **type I**, **type II** and **type III**.

Table : Difference between Type I, Type II and Type III restriction endonucleases

	Type I	Type II	Type III
(i)	Enzyme structure consists of 3 different subunits.	Enzyme structure is simple.	Enzyme structure consists of two different subunits.
(ii)	They require ATP, Mg^{2+} S-adenosyl-methionine for restriction.	They require Mg^{2+} ions for restriction.	They require ATP, Mg^{2+} and S-adenosyl- methionine for restriction.
(iii)	They recognise specific sites within DNA but do not cut these sites.	They recognise specific sites within the DNA and cut these sites.	They recognise specific sites within the DNA but do not cut these sites.

(iv)	They are not used in recombinant DNA technology.	They are used in recombinant DNA technology.	They are not used in recombinant DNA technology.

Nomenclature of Restriction Enzymes :

- (i) Type II restriction enzymes are named for the bacterium from which they have been isolated, For example *Eco*RI enzyme has been isolated from, *Escherichia coli* the bacterium RY13. (ii) The first letter used for the enzyme is the first letter of the bacterium's genus name (in italics), (iii) then comes the first two letter of its species (in italics). (iv) The fourth letter of the name of enzyme is the letter of strain. (v) The end of the name is roman numeral, which indicates the order in which the enzyme was isolated.

Functioning of Restriction Enzyme

- Restriction enzymes are present in many bacteria where they function as a part of their defence mechanism called the **Restriction Modification System.** Molecular basis of this system was first explained by **Wemer Arber in 1965**. The restriction modification system consists of restriction endonucleases and a modification enzyme.
- Synthesising enzymes, DNA ligases, Alkaline phosphatase are other enzymes used in rDNA technology.

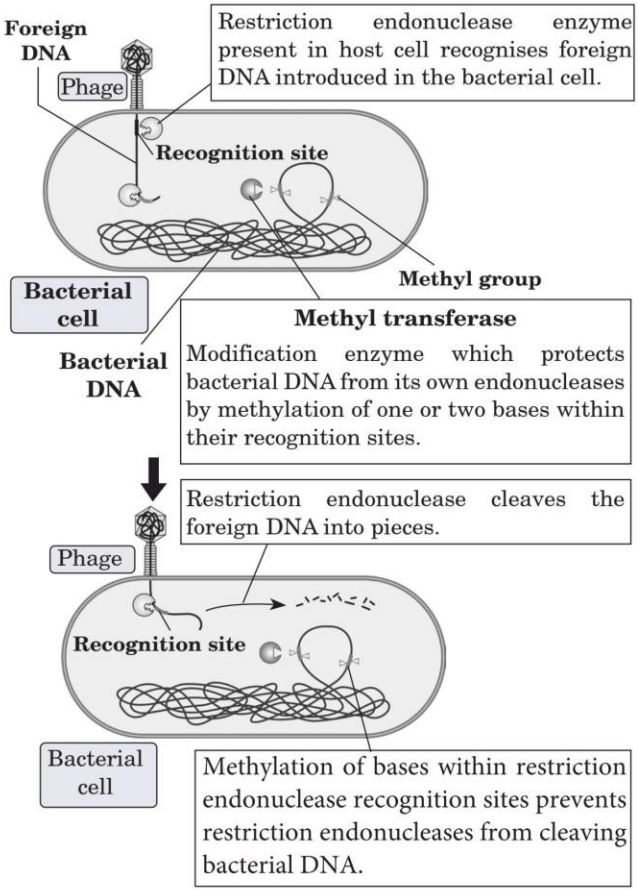


Fig.: Restriction Modification System

CLONING VECTORS (VEHICLE DNA)

- The vectors are DNA molecules that can carry a foreign DNA segment and replicate inside the host cell.
- Vectors may be **plasmids, bacteriophages, cosmids, Yeast Artificial Chromosomes (YACs), Bacterial Artificial Chromosomes (BACs), transposons** and **viruses.**
- Out of these vectors, plasmid vectors and bacteriophage vectors are commonly used.
- Plasmids are extra-chromosomal, self-replicating, usually circular, double-stranded DNA molecules, found naturally in many bacteria and also in some yeast.
- Plasmids are usually not essential for normal cell growth and division, but they often confer some traits to the host organism *e.g.*, resistance to certain antibiotics.
- The most widely used, versatile, easily manipulated vector **pBR322** is an ideal plasmid vector.

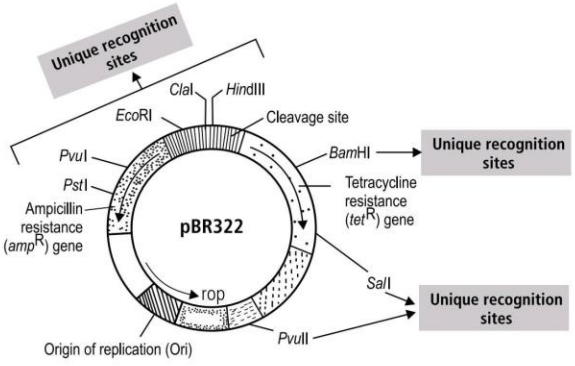


Fig.: *E.coli* Cloning Vector pBR322

- Bacteriophages (a virus that eats upon bacteria) are required for cloning of large DNA fragment.

- Two phages that have been extensively modified for development of cloning vectors are lambda phage and M13 phage.
- Lambda phage vector has a double-stranded, linear DNA genome of 48, 502 bp, in which the 12 bases at each end are unpaired but complementary.
- These vectors allow cloning of DNA fragments up to 23 kb in length.
- M13 phage vector is a filamentous phage which infects *E. coli* having F-pili. Its genome is a single stranded, circular DNA of 6407 bp.
- Some other cloning vectors are cosmid, bacterial Artificial Chromosome (BAC), Yeast Artificial Chromosome (YAC), Phagemid, animal and plant vector, transposon as vector, shuttle vectors.
- The cosmids (like pJC74, pJC720, etc.) can be defined as the hybrid vectors derived from plasmids and phage λ which contain cos site.

Characteristics of a Cloning Vector

The desirable characteristics of a cloning vector are:

- **Origin of replication** (*Ori*) is a sequence from where replication starts. The replication occurs inside the host cells. A prokaryotic DNA has a single origin of replication while eukaryotic DNA may have more than one origin of replication.
- **Selectable markers (antibiotic resistance gene)** are required to identify and eliminate non-transformants and selectively permit the growth of the transformants. Generally, the genes encoding resistance to antibodies such as tetracycline, ampicillin, kanamycin or chloramphenicol, etc., are used as selectable markers for *E.coli*.
- A valuable feature of the cloning vector is the presence of specific **restriction sites** (or **cloning sites**), where the enzyme restriction endonucleases make a cut so that a foreign DNA segment may be introduced (joined) to the vector. This property helps a vector to act as trusted cloning vehicle during gene transfer in genetic engineering. The ligation of alien DNA is carried out at a restriction site present in one of the antibiotic resistance genes.

COMPETENT HOST

- Competent host is essential for transformation with recombinant DNA. Since, DNA is a hydrophilic molecule, it cannot pass through membranes, so bacterial cells must be made capable to take up DNA. This is done by treating them with a specific concentration of a divalent cation, such as calcium ion which increases the efficiency. Recombinant DNA can then be forced into such cells by incubating the cells with recombinant DNA on ice, followed by placing them briefly at 42°C (heat shock) and then putting them back on ice. This enables the bacteria to take up the recombinant DNA. In eukaryotic cells, the term **transformation** is replaced by the term **transfection**.

Vector Mediated Gene Transfer

- Various kinds of vectors *e.g.*, pBR322, λ phage, etc. are used to introduce DNA into many kinds of host cells, including *E. coli,* yeast, animal and plant cells available for genetic engineering. For the expression of some eukaryotic proteins, eukaryotic cells may be the preferred hosts. Yeasts have been used extensively for functional expression of eukaryotic genes because they are the simplest eukaryotic organisms and like bacteria are single celled, genetically well characterised, easy to grow and manipulate.

Vectorless Gene Gransfer

Methods of vectorless gene transfer

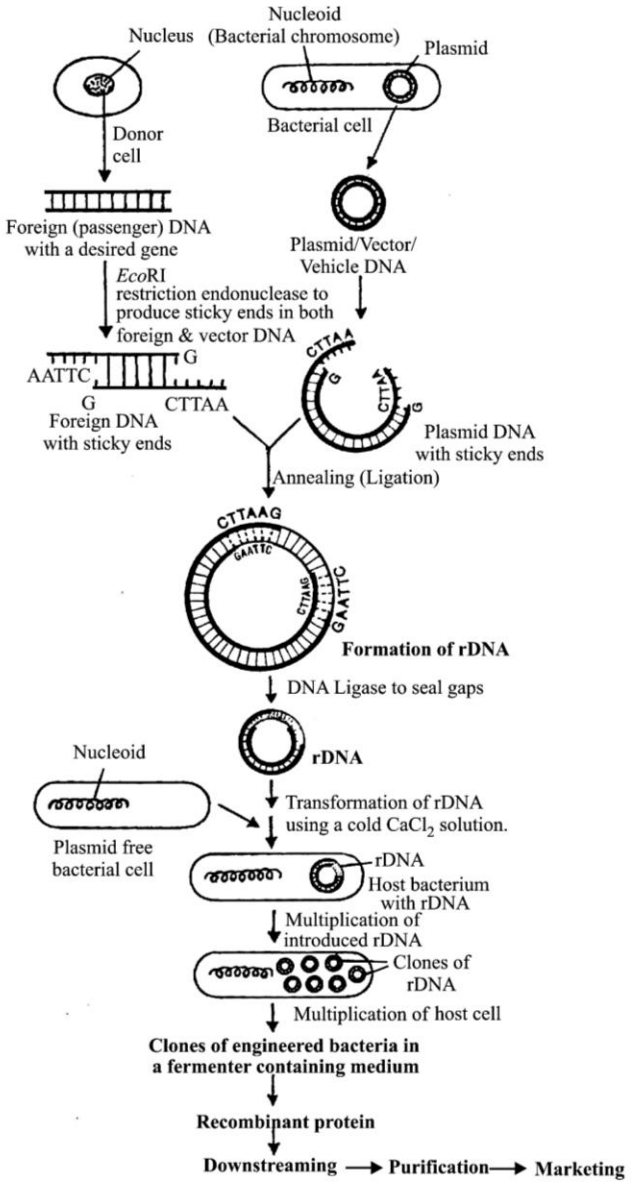
- Different alternative methods have been used to introduce the recombinant DNA into recipient cells of animals without involving carrier molecules.
- **Microinjection :** In this method foreign DNA is directly injected into the nucleus of animal cell or plant cell by using micro needles or micro pipettes. It is used in oocytes, eggs and embryo.

- **Electroporation :** This method involves suspension of cells in a suitable ionic solution containing linearised recombinant plasmid DNA. This mixture is then exposed to low voltage-long pulses or high voltage-short pulses for the desired number of cycles. The electrical pulses are thought to induce transient pores in the plasmalemma through which the DNA molecules are incorporated. Treated cells are then cultured to obtain colonies.
- **Gene gun or biolistic method :**
  - In this method, foreign **gold or tungsten** particles coated with DNA are shooted into the plant cells using a helium pressure particle gun device. This method is also known as **biolistic technique.**
  - Important crop plants like, wheat, rice and maize have now been transformed by this method.
- **Chemical mediated gene transfer :** It involves chemicals such as **polyethylene glycol** that help foreign DNA to enter the host cell.

Processes of Recombinant DNA Technology

- **Isolation of the genetic material (DNA)**
  - In order to cut the DNA with restriction enzymes, it needs to be in pure form, free from other macromolecules.
  - This can be achieved by treating the bacterial cells/plant or animal tissue with enzymes such as **lysozyme (bacteria), cellulase (plant cells), chitinase (fungus)**.
  - The RNA can be removed by treatment with ribonuclease whereas proteins can be removed by treatment with protease.
  - The purified DNA finally precipitates out after the addition of chilled ethanol. This is seen as collection of the threads in suspension.
- **Cutting of DNA at Specific locations**
  - Restriction enzyme digestions are performed by incubating purified DNA molecules with the restriction enzyme, at the optimal conditions for that specific enzyme.
  - Agarose gel electrophoresis is employed to check the progression of a restriction enzyme digestion.
  - **'Gene of interest'** from the source DNA and the **cut vector** with space are mixed and **ligase** is added. This results in the preparation of recombinant *DNA*.
  - If required, gene of interest is amplified using PCR.
- **Insertion of recombinant DNA (rDNA) into host cell/organism**
  - The vector DNA (plasmid DNA) and alien (foreign) DNA carrying gene of interest are cut by the same restriction endonuclease to produce complementary sticky ends. With the help of DNA ligase enzyme, the complementary sticky ends of the two DNAs are joined (annealing) to produce a recombinant (chimaera) DNA.
  - Eukaryotic genes do not function properly when cloned into bacterial cell, because of introns. In such cases, DNA is made from *m*RNA by reverse transcription or synthesised artificially. This rDNA is inserted into host bacterium by **transformation** using cold $CaCl_2$ solution. The bacterial cell containing the desired rDNA is **selected** using selective antibiotic in the culture medium.
- **Obtaining the desirable foreign gene product**
  - When recombinant DNA is transferred into a bacterial, plant or animal cell, the foreign DNA also multiplies. Most of the recombinant technologies are aimed to produce a **desirable protein**.
  - If any protein encoding gene is expressed in a heterologous host it is known as a **"recombinant protein"**.
  - After the cloning of gene of interest one has to maintain the optimum conditions to induce the expression of the target gene and consider producing it on a large scale.

These steps have been summarised in the flow chart :



BIOREACTORS

- Bioreactors are considered as vessels in which raw materials are biologically converted into specific products by microbes, plant and animal cells or their enzymes.
- Small volume cultures cannot give large quantities of the products. To **produce large quantities** of these products, development of bioreactors was required where large volume (100 - 1000 litres) of culture can be processed.
- Bioreactor provides the optimal growth conditions such as temperature, pH, substrate, vitamins, oxygen and salts for obtaining the desired product.
- The most commonly used bioreactors are of **stirring type**. Stirring type bioreactors are
  (i) Simple stirred tank bioreactor and
  (ii) Sparged stirred tank bioreactor
- A **simple stirred tank bioreactor** is a large stainless steel vessel which has the main parts as cooling jacket, air inlet and filter, stirrer, sparger, lines, temperature sensor and control unit.
- In the **sparged stirred-tank bioreactor, sterile air bubbles** are sparged. The surface area for oxygen transfer is hence increased.
- Microorganisms turn raw materials into products such as glucose into alcohol through fermentation. The two basic type of fermentation are possible batch fermentation and continuous culture.
- In batch fermentation nutrients and microbes are put in closed chamber and not changed from outside once the fermentation starts whereas in continuous fermentation nutrients are added continuously and products are taken out as fast as they are made.
- After the formation of product in bioreactors, it undergoes through a series of processes like separation and purification (collectively known as **downstream processing**) before it is ready for marketing as a finished product.
- The downstream processing and quality control testing varies from product to product.

GEL ELECTROPHORESIS

- Electrophoresis is a technique of separation of charged molecules under the influence of an electrical field so that they migrate in the direction of electrode bearing the opposite charge, through a medium/matrix.
- The most commonly used matrix is **agarose** which is a polysaccharide extracted from sea weeds.
- DNA fragments separate according to size through the pores of agarose gel.

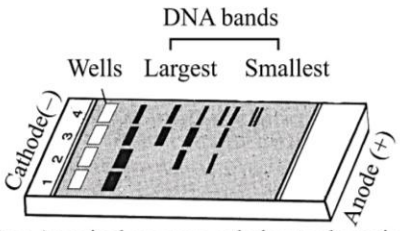


Fig.: A typical agarose gel electrophoresis

- The separated DNA fragments can be seen only after staining the DNA with a compound known as **ethidium bromide** (EtBr) followed by exposure to UV radiation as bright orange coloured bands.
- The separated bands of DNA are cut out from the agarose gel and extracted from the gel piece. This step is called as *elution*.

POLYMERASE CHAIN REACTION (PCR) TECHNIQUE

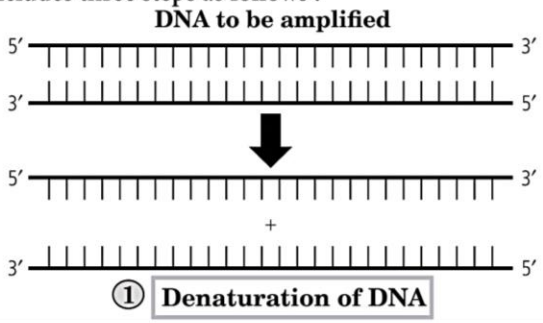
- Polymerase chain reaction (PCR) is a technique of synthesising multiple copies of the desired gene (or DNA) *in vitro*. This technique was developed by **Kary Mullis in 1985**.
- It is based on the principle that a DNA molecule, when subjected to high temperature, splits into two strands due to denaturation.

Basic Requirements of PCR

- **DNA template**. The desired segment of the target DNA molecule that is to be amplified.
- **Two nucleotide primers**. Two nucleotide primers, usually 10-18 nucleotides long and complementary to the sequences present at the 3′ borders of the target DNA segment.
- **Enzyme**. High temperature (more than 90°C) stable DNA polymerase (usually *Taq* polymerase), for synthesis of new DNA molecules.

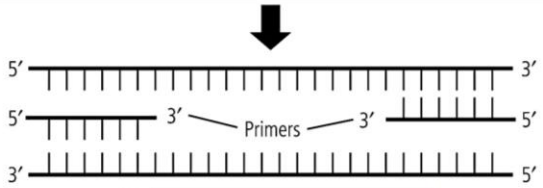
Procedure of PCR

It includes three steps as follows :



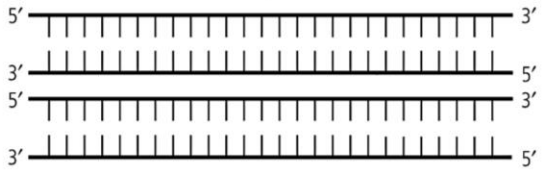
① Denaturation of DNA

The target DNA is first **heated** to a temperature between **94 - 96°C** to ensure DNA denaturation *i.e.*, the separation of the two strands. Each single strand of the target DNA then acts as a template for DNA synthesis.



② Annealing of primers

The mixture is **cooled** to a temperature (of about **40-60°C** that permits annealing of the primer to the complementary sequences in the DNA; these sequences are located at the **3′-ends** of the two strands of the desired segment.



③ Primer extension

The **DNA polymerase** (*Taq* polymerase) synthesises the complementary strands by utilising **NTPs** (deoxynucleoside triphosphates) and Mg^{2+}. The primers are extended towards each other, so that the DNA segment lying between the two primers is copied. This is ensured by employing primers complementary to the 3′-ends of the segment to be amplified. The duration of primer extension is usually **2 min** at **72°C**.

- This process is repeated many times to amplify the required gene of interest. For second cycle, DNA obtained after first cycle is used as template and so on.
- PCR is used in many areas like detection of pathogens, diagnosis of specific mutations, DNA fingerprinting, prenatal diagnosis, etc.

Practice Time

OBJECTIVE TYPE QUESTIONS

Multiple Choice Questions (MCQs)

1. Which one of the following techniques made it possible to genetically engineer living organisms?
(a) Recombinant DNA techniques
(b) X-ray diffraction
(c) Heavier isotope labelling
(d) Hybridisation

2. In genetic fingerprinting, the 'probe' refers to ———
(a) a radioactively labelled single stranded DNA molecule.
(b) a radioactively labelled single stranded RNA molecule.
(c) a radioactively labelled double stranded RNA molecule.
(d) a radioactively labelled double stranded DNA molecule.

3. Following statements describe the characteristics of the enzyme restriction endonuclease. Identify the incorrect statement.
(a) The enzyme recognises a specific palindromic nucleotide sequence in the DNA.
(b) The enzyme cuts DNA molecule at identified position within the DNA.
(c) The enzyme binds DNA at specific sites and cuts only one of the two strands.
(d) The enzyme cuts the sugar-phosphate backbone at specific sites on each strand.

4. A selectable marker is used to
(a) help in eliminating the non-transformants, so that the transformants can be regenerated.
(b) identify the gene for a desired trait in an alien organism.
(c) select a suitable vector for transformation in a specific crop.
(d) mark a gene on a chromosome for isolation using restriction enzyme.

5. Given below are four statements pertaining to separation of DNA fragments using gel electrophoresis. Identify the incorrect statements.
(i) DNA is negatively charged molecule and so it is loaded on gel towards the anode terminal.
(ii) DNA fragments travel along the surface of the gel whose concentration does not affect movement of DNA.
(iii) Smaller the size of DNA fragment larger is the distance it travels through it.
(iv) Pure DNA can be visualized directly by exposing UV radiation.
Choose the answer from the options given below.
(a) (i), (iii) and (iv) (b) (i), (ii) and (iii)
(c) (ii), (iii) and (iv) (d) (i), (ii) and (iv)

6. The DNA fragments separated on an agarose gel can be visualised after staining with
(a) acetocarmine (b) aniline blue
(c) ethidium bromide (d) bromophenol blue.

7. What is the criterion for DNA fragments movement on agarose gel during gel electrophoresis ?
(a) The smaller the fragment size, the farther it moves.
(b) Positively charged fragments move to farther end.
(c) Negatively charged fragments do not move.
(d) The larger the fragment size, the farther it moves.

8. Which of the following restriction enzymes produces blunt ends?
(a) *Sal*I (b) *Eco*RV
(c) *Xho*I (d) *Hin*dIII

9. Which of the following is a restriction endonuclease?
(a) DNaseI (b) RNase
(c) *Hin*dII (d) Protease

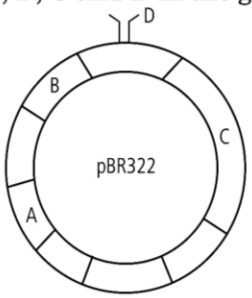
10. Which of the following is not a feature of the plasmids?
(a) Transferable (b) Single-stranded
(c) Independent replication
(d) Circular structure

11. Most suitable method of introducing alien DNA into a plant cell is
(a) lipofection (b) biolistics
(c) heat shock method (d) microinjection.

12. The DNA molecule to which the gene of interest is integrated for cloning is called
(a) template (b) carrier
(c) transformer (d) vector.

13. Restriction endonucleases are
(a) used for *in vitro* DNA synthesis
(b) synthesised by bacteria as part of their defence mechanism
(c) present in mammalian cells for degradation of DNA when the cell dies
(d) used in genetic engineering for ligating two DNA molecules.

14. Identify A, B, C and D in the given diagram.



(a) A-*ori*, B-*amp*^{R}, C-*tet*^{R}, D-*Hin*dIII
(b) A-*Hin*dIII, B-*tet*^{R}, C-*amp*^{R}, D-*ori*
(c) A-*amp*^{R}, B-*tet*^{R}, C-*Hin*dIII, D-*ori*
(d) A-*tet*^{R}, B-*Hin*dIII, C-ori, D-*amp*^{R}

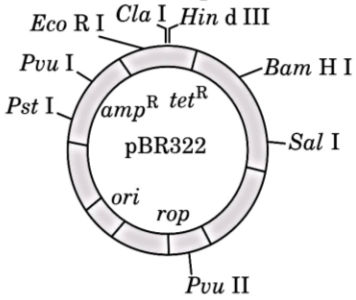
15. Select the wrong statement.
(a) The presence of chromogenic substrate gives blue colour colonies, if the plasmid in the bacteria does not have an insert.
(b) Retroviruses in animals have the ability to transform normal cells into cancerous cells.
(c) In microinjection, cells are bombarded with high velocity microparticles of gold or tungsten coated with DNA.
(d) Since DNA is a hydrophilic molecule it cannot pass through cell membranes.

16. Which vector can clone only a small fragment of DNA?
(a) Bacterial artificial chromosome
(b) Yeast artificial chromosome
(c) Plasmid
(d) Cosmid

17. Identify the DNA segment which is not a palindromic sequence.
(a) 5′ GGATCC 3′ (b) 5′ GAATTC 3′
3′ GGTACC 5′ 3′ CTTAAG 5′
(c) 5′ GCGGCCGC 3′ (d) 5′ CCCGGG 3′
3′ CGCCGGCG 5′ 3′ GGGCCC 5′

18. The colonies of recombinant bacteria appear white in contrast to blue colonies of non-recombinant bacteria because of
(a) insertional inactivation of alpha galactosidase in recombinant bacteria
(b) inactivation of glycosidase enzyme in recombinant bacteria
(c) non-recombinant bacteria containing beta galactosidase
(d) insertional inactivation of alpha galactosidase in non-recombinant bacteria.

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



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

20. For transformation, micro-particles coated with DNA to be bombarded with gene gun are made up of
(a) silver or platinum (b) platinum or zinc
(c) silicon or platinum (d) gold or tungsten.

21. The enzymes which are absolutely necessary for recombinant DNA technology are
(a) restriction endonucleases and topoisomerases
(b) endonucleases and polymerases
(c) restriction endonucleases and ligases
(d) peptidases and ligases.

22. Given below is a sample of a portion of DNA strand giving the base sequence on the opposite strands. What is so special shown in it?
5′_____ GAATTC′ _____ 3′
3′_____ CTTAAG _____ 5′
(a) Replication completed
(b) Deletion mutation
(c) Start codon at the 5′ end
(d) Palindromic sequence of base pairs.

23. There is a restriction endonuclease called *Eco*RI. What does "*co*" part in it stand for?
(a) Colon (b) Coelom
(c) Coenzyme (d) Coli

24. Agarose extracted from sea weeds is used in
(a) spectrophotometry (b) tissue culture
(c) PCR (d) gel electrophoresis.

25. In genetic engineering, a DNA segment (gene) of interest, is transferred to the host cell through a vector. Consider the following four agents (i-iv) in this regard and select the correct option about which one or more of these can be used as a vector/vectors.
(i) Bacterium (ii) Plasmid
(iii) *Plasmodium* (iv) Bacteriophage
(a) (i), (ii) & (iv) (b) (i) only
(c) (i) & (iii) (d) (ii) & (iv)

26. Which one of the following is used as vector for cloning genes into higher organisms?
(a) Baculovirus
(b) *Salmonella typhimurium*
(c) *Rhizopus nigricans*
(d) Retrovirus

27. DNA precipitation out of a mixture of biomolecules can be achieved by treatment with
(a) chilled chloroform (b) isopropanol
(c) chilled ethanol
(d) methanol at room temperature.

28. Which one of the following equipments is essentially required for growing microbes on a large scale, for industrial production of enzymes?
(a) Bioreactor (b) BOD incubator
(c) Sludge digester (d) Industrial oven

29. The process of separation and purification of expressed protein before marketing is called
(a) downstream processing
(b) bioprocessing
(c) postproduction processing
(d) upstream processing.

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

31. Match the items in column I with their uses in column II and choose the right option.

Column I	Column II
A. ELISA	(i) Antigen-antibody interaction
B. PCR	(ii) Gene amplification
C. Biolistics	(iii) Direct introduction of recombinant DNA
D. Micro-injection	(iv) Gold coated DNA

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

32. Match the items in column I with their uses in column II and choose the right option.

Column I	Column II
A. *Bacillus thuringiensis*	(i) Restriction endonuclease
B. *Agrobacterium tumefaciens*	(ii) Thermostable DNA polymerase
C. *Thermus aquaticus*	(iii) Insecticidal protein
D. *Escherichia coli*	(iv) Ti plasmid

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

33. Elution means
(a) making the DNA bands visible under UV radiation
(b) separation of DNA fragments on agarose gel
(c) isolating alien DNA from the choice organism
(d) cutting and extraction of DNA bands from the agarose gel.

34. *In vitro* clonal propagation in plants is characterised by
(a) PCR and RAPD
(b) northern blotting
(c) electrophoresis and HPLC
(d) microscopy.

35. Identify the desirable characteristics for a plasmid used in rDNA technology from the following.
A. Ability to multiply and express outside the host in a bioreactor
B. A highly active promoter
C. A site at which replication can be initiated
D. One or more identifiable marker genes
E. One or more unique restriction sites

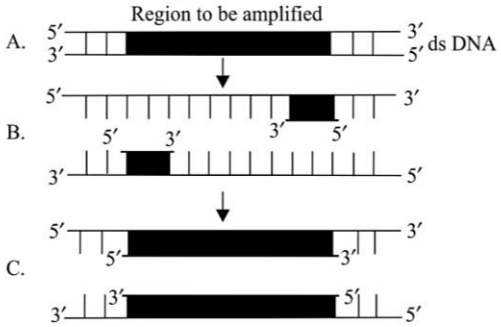
(a) A, C, D and E only (b) A, C and E only
(c) B, C, D and E only (d) B, C and E only

36. Match the entries in column I with those of column II and choose the correct answer.

	Column I		Column II
A.	Restriction endo-nucleases	(p)	Kohler and Milstein
B.	Polymerase chain reaction	(q)	Alec Jeffreys
C.	DNA fingerprinting	(r)	Arber
D.	Monoclonal antibodies	(s)	Karry Mullis

(a) A - (r), B - (s), C - (q), D - (p)
(b) A - (r), B - (q), C - (s), D - (p)
(c) A - (q), B - (r), C - (s), D - (p)
(d) A - (q), B - (s), C - (r), D - (p)

37. The figure below shows three steps (A, B, C) of Polymerase Chain Reaction (PCR). Select the option giving correct identification together with what it represents?



(a) B - Denaturation at a temperature of about 98°C separating the two DNA strands.
(b) A - Denaturation at a temperature of about 50°C.
(c) C - Extension in the presence of heat stable DNA polymerase.
(d) A - Annealing with two sets of primers.

38. Which one is a true statement regarding DNA polymerase used in PCR?

(a) It is used to ligate introduced DNA in recipient cells.
(b) It serves as a selectable marker.
(c) It is isolated from a virus.
(d) It remains active at high temperature.

39. PCR and restriction fragment length polymorphism are the methods for

(a) study of enzymes
(b) genetic transformation
(c) DNA sequencing
(d) genetic fingerprinting.

40. The term, 'Southern Blotting' refers to

(a) transfer of DNA fragments from *in vitro* cellulose membrane to electrophoretic gel
(b) attachment of probes to DNA fragments
(c) transfer of DNA fragments from electrophoretic gel to nitrocellulose sheet
(d) comparison of DNA fragments from two sources.

Case Based MCQs

Case I : Read the following passage and answer any four questions from 41 to 45 given below:

Rama lives in a society where a robbery occurred last night. Robbers came into the flat and murdered the old lady residing there. Police came and restricted the entry into the flat. They took samples from the room, where the dead body was found. While examining, they found that there is some blood and tissue in the nails of old lady. According to their observation, police filtered out their inspection to three suspects *viz.* servant, cook and milkman. Finally after two days of robbery, police caught the criminal. It was the old lady's cook. Rama was amazed to see that how quickly police completed and shut the case. She asked the inspector that how they did it? The police man told her that it become possible due to the sample collected from the victim, that lead them to the criminal. The sample taken from nail scraping was amplified using PCR and then tested.

41. What technique was used by the police to identify the criminal?

(a) DNA fingerprinting (b) Gel electrophoresis
(c) Molecular diagnosis (d) Cloning

42. In PCR, the temperature used to denature the DNA is about

(a) 76°C (b) 25°C
(c) 95°C (d) 40°C.

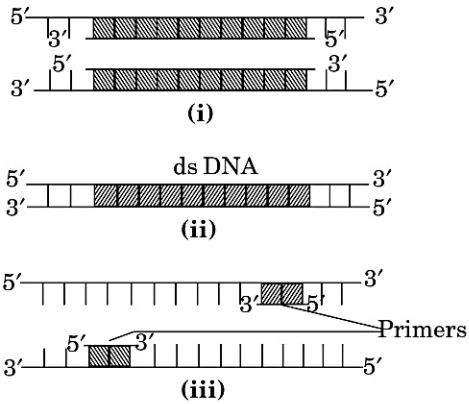
43. Which of the following statements regarding PCR is correct?

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

(b) With the help of DNA ligase, the complementary sticky ends of the DNA are joined to produce a *r*DNA.
(c) Since the sequence of primers are complementary to 5′ end of the template DNA, they anneal to it.
(d) DNA purified from the cell is precipitated by adding hot ethanol.

44. Taq polymerase synthesises DNA region between the primers using
(a) Mg^{2+} (b) dNTPs
(c) DNA ligase (d) both (a) and (b).

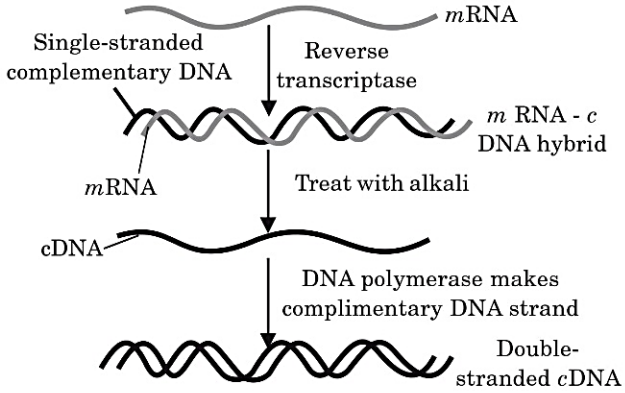
45. Given below are steps of polymerase chain reaction.



Select the option that correctly mention the sequence in which they occur.
(a) (ii) (iii) (i) (b) (i) (ii) (iii)
(c) (iii) (i) (ii) (d) (ii) (i) (iii)

Case II : Read the following passage and answer any four questions from 46 to 50 given below:

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



46. Reverse transcriptase enzyme was discovered by
(a) Temin and Baltimore
(b) Cohen and Boyer
(c) Arber and Nathan (d) Paul Berg.

47. During *c*DNA formation, what would happen if DNA formed by reverse transcriptase is not treated with the alkali?
(a) *c*DNA will not be digested
(b) *m*RNA will not be digested
(c) Hydrogen bonds will not form between base pairs
(d) *m*RNA will not be formed.

48. Enzyme that helps in the formation of double stranded *c*DNA is
(a) DNA synthetase (b) ligase
(c) DNA polymerase (d) helicase.

49. DNA polymerase can be obtained form
(a) retrovirus (b) *Agrobacterium*
(c) tobacco mosaic virus
(d) *Thermus aquaticus.*

50. DNA synthesised without a template is referred to as
(a) complementary DNA
(b) random DNA
(c) synthetic DNA (d) Z-DNA.

Assertion & Reasoning Based MCQs

For question numbers 51-60, two statements are given-one labelled Assertion and the other labelled Reason. Select the correct answer to these questions from the codes (a), (b), (c) and (d) as given below.

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

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

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

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

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

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

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

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

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

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

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

SUBJECTIVE TYPE QUESTIONS

Very Short Answer Type Questions (VSA)

1. Who is considered as the "father of genetic engineering"?
2. Name the host cells in which microinjection technique is used to introduce an alien DNA.
3. What is the role of sterile air bubbles in the sparged stirred-tank bioreactor?
4. Mention the source of thermostable DNA polymerase.
5. Name the material used as matrix in gel-electrophoresis and mention its role.
6. What are synthesising enzymes?
7. State what happens when an alien gene is ligated at *Sal*I site of pBR322 plasmid.
8. What are the drawbacks of stirred tank bioreactors?
9. Why do DNA fragments move towards the anode during gel-electrophoresis?
10. During the isolation of DNA, how can you remove RNA and protein from the DNA solution?

Short Answer Type Questions (SA-I)

11. Why is 'plasmid' an important tool in biotechnology experiments?
12. Write the role of '*ori*' and 'restriction' site in a cloning vector pBR322.

13. Name the natural source of agarose. Mention one role of agarose in biotechnology.

14. What are the two main discoveries that led to the built of genetic engineering ?

15. A recombinant DNA is formed when sticky ends of vector DNA and foreign DNA join. Explain how the sticky ends are formed and get joined.

16. Why is it essential to maintain sterile condition in biotechnological processes?

17. Explain any two methods of vectorless gene transfer.

18. State the role of UV-light and ethidium bromide during gel electrophoresis of DNA fragments.

19. Rearrange the following in the correct sequence to accomplish an important biotechnological reaction :

(i) Denaturation of ds-DNA

(ii) Chemically synthesised oligonucleotides

(iii) Primers

(iv) Complementary region of DNA

(v) Thermostable DNA polymerase (from *Thermus aquaticus)*

(vi) Nucleotides provided

(vii) Genomic DNA template

(viii) *In vitro* synthesis of copies of DNA of interest

(ix) Enzyme DNA-polymerase.

20. Why are genes encoding resistance to antibiotics considered useful selectable markers for *E. coli* cloning vector? Explain with the help of one example.

Short Answer Type Questions (SA-II)

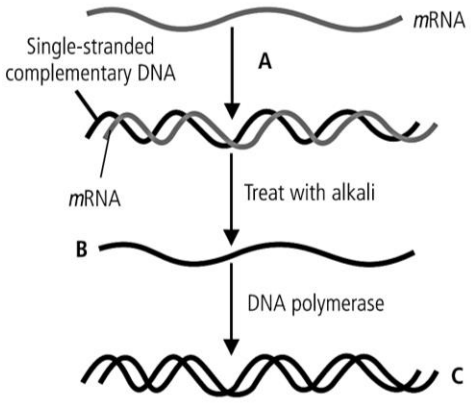
21. Prepare a flow chart in formation of recombinant DNA by the action of restriction endonuclease enzyme *Eco*RI.

22. Refer to the given figure and answer the following questions.

(a) Identify the labelled part A and mention its function.

(b) What does B and C represent?

(c) Briefly describe the process shown in the given figure.



23. Differentiate between rDNA and cDNA.

24. How does β-galactosidase coding sequence act as a selectable marker? Why is it a preferred selectable marker to antibiotic resistance genes? Explain.

25. What are bioreactors ? List five growth conditions that a bioreactor provides for obtaining the desired product.

26. How and why is the bacterium *Thermus aquaticus* employed in recombinant DNA technology ? Explain.

27. Give reasons why:

(a) DNA cannot pass into a host cell through the cell membrane.

(b) Proteases are added during isolation of DNA for genetic engineering.

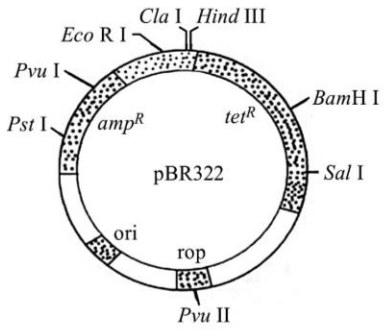
(c) Single cloning site is preferred in a vector.

28. (a) Why must a cell be made 'competent' in biotechnology experiments? How does calcium ion help in doing so?

(b) State the role of 'biolistic gun' in biotechnology experiments.

29. Name the technique to obtain multiple copies, of a DNA segment of interest, synthesized *in vitro*. Name two sets of primers that are necessary for reaction to occur. Mention three diagnostic applications of this technique.

30. Explain the importance of **(i)** *ori*, **(ii)** amp^R and **(iii)** *rop* in the *E.coli* vector shown below.



31. Explain the role of Ti plasmids in biotechnology.

32. **(a)** Mention the difference in the mode of action of exonuclease and endonuclease.

(b) How does restriction endonuclease function?

33. Name the source organism from which Ti plasmid is isolated. Explain the use of this plasmid in biotechnology.

34. **(a)** What is *Eco*RI? What does 'R' represent in this?

(b) Give the palindromic nucleotide sequence recognised by it.

(c) Explain its action.

35. Read the following base sequence of a certain DNA strand and answer the questions that follow:

	A	A	G	A	A	T	T	C	A	A		
	T	T	C	T	T	A	A	G	T	T		

(i) What is called a 'palindromic sequence' in a DNA ?

(ii) Write the palindromic nucleotide sequence shown in the DNA strand given and mention the enzyme that will recognise such a sequence.

(iii) State the significance of enzymes that identify palindromic nucleotide sequences.

Long Answer Type Questions (LA)

36. What is cloning vector ? Why is it used ? Explain the technique of using such a vector in *E.coli*.

37. If a desired gene is identified in an organism for some experiments, explain the process of the following :

(a) Cutting of desired gene at specific locations.

(b) Synthesis of multiple copies of the desired gene.

38. **(a)** Mention the role of vectors in recombinant DNA technology. Given any two examples.

(b) With the help of diagrammatic representation only, show the steps of recombinant DNA technology.

ANSWERS

OBJECTIVE TYPE QUESTIONS

1. **(a)**

2. **(a) :** For DNA fingerprinting special single stranded DNA-probes are made in the laboratory. DNA-probes contain repeated sequences of bases complementary to those on VNTRs. These probes are made radioactive by labelling with radioactive isotopes. This step helps in detecting DNA fingerprints or variable number of tandem repeats (VNTRs).

3. **(c) :** The restriction endonuclease enzyme inspects the length of a DNA sequence. Once it recognises specific sequence, it binds to the DNA and cuts each of the two strands of the double helix at specific points in their sugar phosphate backbone. Special sequence in the DNA recognised by restriction endonuclease is called palindromic nucleotide sequence.

4. **(a)** **5.** **(d)**

6. **(c) :** The separated DNA fragments can be seen only after staining them with a compound known as ethidium bromide (EtBr) followed by exposure to UV radiation as bright orange coloured bands.

7. **(a) :** Electrophoresis is a technique used for the separation of substances of different ionic properties. Since the DNA fragments are negatively charged molecules, they can be separated by allowing them to move towards the anode. DNA fragments move towards the anode according to their molecule size through the pores of agarose gel. Thus, the smaller fragments move farther away as compared to larger fragments.

8. **(b) :** *Eco*RV is a type II restriction endonuclease isolated from certain strains of *E.coli*. It creates blunt ends. It recognises the palindromic sequence of 6 bases as shown here:

Restriction site

5′ GAT ATC 3′
3′ CTA TAG 5′

*Sal*I, *Xho*I and *Hind*III restriction enzymes produce sticky ends.

9. (c) : *Hind*II is the first restriction endonuclease. It was isolated from *Haemophilus influenzae* Rd. It always cut DNA at specific position producing blunt ends. DNase I is an endonuclease that cleaves DNA preferentially at phosphodiester linkages adjacent to a pyrimidine nucleotide non-specially. RNase is a type of nuclease that catalyses the degradation of RNA into smaller components. It can be endoribonuclease or exoribonuclease. A protease is an enzyme that perform proteolysis, *i.e.,* protein catabolism by hydrolysis of the peptide bonds.

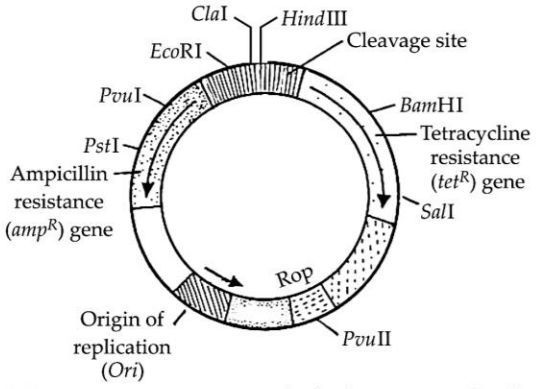
10. (b) : Plasmids are extra-chromosomal, self-replicating, usually circular, double-stranded DNA molecules that serve as vectors which carry foreign DNA segment and replicate inside host cell.

11. (b) : Biolistics technique also known as ballistic method of DNA delivery has general applicability to plant species and can be used to deliver DNA into virtually all tissues.

12. (d) : Vector is a DNA molecule that carries a foreign DNA segment and replicates inside a host cell. The vector DNA and foreign DNA carrying gene of interest are cut by the same restriction endonuclease enzyme to produce complementary sticky ends. With the help of DNA ligase enzyme, the complementary sticky ends of the two DNAs are joined to produce a recombinant DNA (rDNA), which is then introduced into the host cell.

13. (b)

14. (a) : pBR322 is an artificial cloning vector whose essential features have been shown in the following diagram:



15. (c) : In microinjection method of DNA transfer, foreign DNA is directly injected into the nucleus of animal cell or plant cell by using micro needles or micro pipettes. It is used in oocytes, eggs and embryo. In gene gun method tungsten or gold particles coated with foreign DNA are bombarded into target cell at very high velocity.

16. (c)

17. (a) : Palindromic nucleotide sequence in the DNA molecule is the sequence of base pairs that reads same when orientation of reading is same, *i.e.*, 5′ to 3′ on both strands or 3′ to 5′ on both strands. Hence, (a) is the correct answer, since it does not read same from both the sides.

18. (c) : The presence of restriction sites within the markers tet^r and amp^r of plasmid permits an easy selection for cells transformed with recombinant plasmid. Insertion of the DNA fragment into the plasmid makes antibiotic resistance genes nonfunctional, for example, insertion of the DNA fragment into the plasmid (pBR322) using *Pst* I or *Pvu* I makes amp^r nonfunctional. Bacterial cells containing such a recombinant pBR322 will be unable to grow in the presence of ampicillin, but will grow on tetracycline. This process, however, becomes burdensome because it requires simultaneous plating on two plates having different antibiotics. Thus, alternative selectable marker is developed to differentiate recombinants and non-recombinants on the basis of their ability to produce colour in the presence of a chromogenic substance. Here, a recombinant DNA is inserted in the coding sequence of an enzyme β-galactosidase. pUC18 plasmid contains this gene which allows it to produce β-galactosidase which degrades certain sugars and produces a blue pigment when exposed to specific substrate analog. If the plasmid in the bacterium does not have an insert, *i.e.*, is non-recombinant, the presence of chromogenic substrate gives blue coloured colonies. Presence of insert in the plasmid in recombinant bacterium does not produce any colour, such bacterial colonies are marked as recombinant colonies.

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

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

21. (c) : An important step in the process of genetic engineering is the preparation of recombinant DNA (rDNA). For this, the vector DNA and foreign DNA (carrying gene of interest) are needed to be cut at specific sites and joined to produce rDNA. This is achieved by enzymes, restriction endonucleases and DNA ligases. Restriction endonucleases act as molecular scissors or chemical scalpels which recognize the base sequence at palindrome sites in DNA duplex (a section of dsDNA having sequence of bases on one strand inverted and repeated on the other) and cut each of the two strands of the double helix at specific points in their sugar phosphate

backbones. DNA ligases are known as genetic gum as they form phosphodiester bonds between adjacent nucleotides and covalently link two individual fragments of dsDNA.

22. (d)

23. (d) : The enzyme restriction endonuclease *Eco*RI is found in the colon bacteria *E. coli*. So, here '*co*' stands for coli. According to nomenclature of restriction enzyme, the first letter used for the enzyme is the first letter of the genus name (in italics) of the bacterium, then comes the first two letters of its species (also in italics), next is the strain of the organism. At last is a Roman numeral signifying the order of discovery. Here, the enzyme *Eco*RI was isolated from the bacterium *Escherichia coli* (co), strain RY13(R) and it was first endonuclease (I) isolated from *E.coli*.

24. (d)

25. (d) : Plasmid and bacteriophage are used as vectors in genetic engineering. Plasmid is an autonomously replicating circular extra chromosomal DNA found in bacteria. They can be transferred from cell to cell in a bacterial colony. This characteristic is being used in biotechnology for transferring desirable gene into target gene of the host. Whereas, bacteriophage is a bacterial virus which can infect it, quickly multiply within and destroy their host (virus) cells. During infection bacteriophages inject their DNA into these cells. The injected DNA selectively replicate and are expressed in the host that results in a multiplication of phages that ultimately burst out of the cell (by lysis). This ability of transferring DNA from the phage genome to specific host during infection process gave scientists the idea that specially designed phage vectors could be used for gene cloning.

26. (d) : Retroviruses in animals have the ability to transform normal cells into cancerous cells. We have transformed these pathogens into useful vectors for delivering genes of interest to humans. Retroviruses have been disarmed and are now used to deliver desirable genes into animal cells. So, once a gene or a DNA fragment has been ligated into a suitable retroviral vector it is transferred into a bacterial, plant or animal host (where it multiplies).

27. (c) : In order to cu t the DNA with restriction enzymes, it needs to be in pure form, free from other macromolecules. Since the DNA is enclosed by the membranes, we have to break the cell open to release DNA and other macromolecules like RNA, proteins, polysaccharides and lipids. It is obtained by treating the bacterial cells/plant or animal tissue with enzymes. Other molecules are removed by appropriate treatments and purified DNA ultimately precipitates out after the addition of chilled ethanol.

28. (a)

29. (a) : After the formation of the product in the bioreactor, it undergoes some processes before a finished product is ready for marketing. The process includes separation and purification of products which are collectively called downstream processing. The product obtained is subjected to quality control, testing and kept in suitable preservatives.

30. (c) : A stirred-tank reactor is usually cylindrical or with a curved base to facilitate the mixing of the reactor contents. The stirrer facilitates, even mixing and oxygen availability throughout the bioreactor.

31. (a)

32. (c)

33. (d)

34. (a) : Clonal propagation represents the technique in which vegetative tissue is used to produce plants that are genetically identical to their parents. It provides rapid vegetative multiplication of plant material for agriculture, horticulture and forestry. It can be characterized by PCR and RAPD. The polymerase chain reaction (PCR) technique, generates microgram (mg) quantities of DNA copies (upto billion copies) of the desired DNA (or RNA) segment, present even as a single copy in the initial preparation, in a matter of few hours. RAPD stands for Random Amplification of Polymorphic DNA. It is a type of PCR, but the segments of DNA that are amplified are random. No knowledge of the DNA sequence for the targeted gene is required, as the primers will bind somewhere in the sequence, but it is not certain exactly where. Its resolving power is much lower than targeted, species specific DNA comparison methods, such as short tandem repeats.

35. (c) : Plasmids are extrachromosomal DNA present naturally in a bacterial cell. They are small double-stranded and closed circular DNA. They are used in recombinant DNA technology as cloning and expression vectors. For acting as a vehicle for the desired gene, they should be able to multiply inside the host cell thus need a replication origin site, should have marker genes and unique restriction sites.

36. (a)

37. (c) : At the start of polymerase chain reaction (PCR), the DNA from which a segment is to be amplified, an excess of the two primer molecules, the four deoxynucleoside triphosphates and the heat stable DNA polymerase, *i.e.*, *Taq* polymerase are mixed together in the reaction mixture that has appropriate quantities of Mg^{2+}. The following operations are now performed sequentially.

(i) Denaturation

The reaction mixture is first heated to a temperature between 90 - 98°C (commonly 94°C) that ensures DNA denaturation *i.e.*, the separation of the two strands. Each single strand of the target DNA then acts as a template for DNA synthesis.

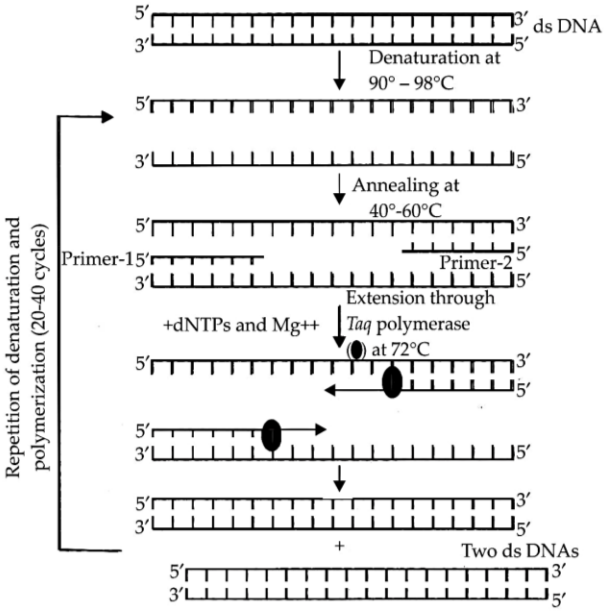
(ii) Primer annealing

The mixture is now cooled to a temperature (generally 40-60°C) that permits annealing of the primer to the complementary sequences in the DNA; these sequences are located at the 3′-ends of the two strands of the desired segment. This step is called annealing.

(iii) Primer extension

The temperature is now so adjusted that the DNA polymerase synthesises the complementary strands by utilising 3′–OH of the primers. This reaction is the same as that occurs *in vivo* during replication of the leading strand of a DNA duplex. The primers are extended towards each other so that the DNA segment lying between the two primers is copied, this is ensured by employing primers complementary to the 3′-ends of the segment to be amplified. The duration of primer extension is usually 2 minute at 72°C.

A schematic representation of PCR can be illustrated as follow.



38. (d) : Thermostable *taq* polymerase, a thermostable DNA polymerase obtained from bacterium *Thermus aquaticus* which remains active during high temperature induced denaturation of DNA during PCR.

39. (d) : Polymerase chain reaction (PCR) is used to amplify a small DNA fragment to obtain its large quantity. PCR is very helpful in DNA fingerprinting in such cases where the culprit has to be identified from a very small blood, semen or other cell sample from a crime scene. RFLP (Restriction fragment length polymorphism) corresponds to the occurrence of different cleavage sites for restriction enzymes in the DNA of different individuals of the same species. RFLPs have provided geneticists with a powerful set of genetic markers for gene mapping and gene tracking. It is used in DNA fingerprinting.

40. (c) : Southern blotting is a chromatographic technique for isolating and identifying specific fragments of DNA, such as the fragments formed as a result of DNA cleavage by restriction enzymes. The mixture of fragments is subjected to electrophoresis through an agarose gel, followed by denaturation to form single-stranded fragments. These are transferred, or 'blotted', onto a nitro-cellulose filter where they are immobilized in their relative positions. Specific gene probes labelled with a radioisotope or fluorescent marker are then added. These hybridize with any complementary fragments on the filter, which are subsequently revealed by autoradiography or a fluorescence detector.

41. (a) : DNA fingerprinting is one of highly accurate application of biotechnology. It is helpful in solving crime, legal disputes, establishing identity of criminal or parents, etc.

42. (c) In PCR, during denaturation, the target DNA is heated at high temperature resulting in the separation of the two strands.

43. (b) : Taq polymerase isolated from bacterium *Thermus aquaticus* is stable at high temperature. Sequence of primers are complementary to 3′ end of the template. Purified DNA is precipitated by adding chilled ethanol.

44. (d)

45. (a) : Three steps of PCR are : denaturation, annealing and extension.

46. (a)

47. (b) : The cDNA formation involves the alkaline denaturation of the *m*RNA-cDNA hybrid. The double stranded DNA molecule formed after the activity of reverse transcriptase is treated with alkali to digest *m*RNA.

48. (c) : A cDNA strand is formed on the separated single stranded DNA template with the help of DNA polymerase enzyme.

49. (d)

50. (c)

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

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

53. (a) **54. (b)**

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

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

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

58. (a) : Bacteriophage vector have two advantages over plasmid vectors.

(i) They are more efficient than plasmids for the cloning of large DNA fragments. For example, the largest cloned insert in lambda phage is 24kb while for plasmid vector it is less than 15 kb.

(ii) It is easier to screen a large number of phage plaques than bacterial colonies for identification of recombinant vectors.

59. (b)

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

SUBJECTIVE TYPE QUESTIONS

1. Genetic engineering was started by Paul Berg (1972) when he was able to introduce a gene of SV-40 into a bacterium. He is often considered as "father of genetic engineering".

2. Microinjection technique is used to introduce an alien DNA directly into the nucleus of the animal host cells such as oocytes, eggs and embryo.

3. The sterile air bubbles in the sparged stirred-tank bioreactor increases the surface area for oxygen transfer.

4. Thermophilic bacterium *Thermus aquaticus* is the source of thermostable DNA *Taq* polymerase.

5. Most commonly used matrix in DNA gel electrophoresis is agarose. It provides sieving effect for separation of DNA fragments according to their size.

6. The enzymes that are used to synthesise DNA strands on suitable templates are called synthesising enzymes. They are of two types : DNA polymerase and reverse transcriptase.

7. When an alien gene is ligated at *SalI* site of pBR322, the gene tet^R becomes non functional and plasmid loses its tetracycline resistance. Hence, the cell possessing such recombinant pBR322 will not be able to grow on tetracycline.

8. The main drawback of stirred-tank bioreactor is that it is relatively expensive to run, which is mainly due to high energy requirements.

9. DNA is a negatively charged molecule hence during gel electrophoresis it moves towards anode (positive electrode) under the influence of electrical field.

10. RNA can be removed by treatment with ribonuclease whereas proteins can be removed by treatment with protease.

11. Plasmid have the ability to replicate within bacterial cells independent of the control of chromosomal DNA and have high copy number, therefore any alien DNA ligated to it, also multiplies to equal the copy number of plasmids. So, it is used as a vector in gene cloning experiments and thus, plays a role of an important tool in biotechnology.

12. Origin of replication (*ori*) site in cloning vector pBR322 is a sequence from where replication starts. Any piece of DNA when linked to this sequence can be made to replicate within host cell. Restriction site within the markers tet^R and amp^R genes permit an easy selection for cells transformed or the recombinant pBR322.

13. Agarose is commonly used as matrix in agarose gel electrophoresis. It is extracted from sea weeds. In recombinant DNA technology, agarose is used to separate DNA fragments according to their sizes.

14. Genetic engineering is the technique to form recombinant DNA and then to introduce recombinant DNA into appropriate host. Two main discoveries which led to genetic engineering are :

(i) Discovery of plasmid by William Hays and Joshua Lederberg in 1952.

(ii) Discovery of restriction endonuclease by Arber in 1962.

15. When restriction enzymes cut the strand of DNA a little away from the centre of the palindromic sites, between the same two bases on the opposite strands, it leaves single stranded portions at the ends. This forms overhanging stretches called sticky ends on each strand. They are called sticky as they form hydrogen bonds with their complementary cut counterparts. The stickiness of the ends facilitates the action of the enzyme DNA ligase.

16. Sterileconditionenablegrowthofonlythedesiredmicrobe/eukaryotic cell in large quantities for the biotechnological products like antibiotics, enzymes, etc.

17. Vectorless gene transfer is a method to introduce recombinant DNA into recipient cells of host without involving carrier molecule.

Two methods of vectorless gene transfer are:

(i) Microinjection : It is the introduction of foreign gene into plant cell or animal cell by using microneedles or micropipettes.

(ii) Electroporation : In this method, electrical impulses induce transient pores in the plant cell membrane through which the DNA molecules are incorporated into the plant cells.

18. DNA fragments can be seen only after staining. Ethidium bromide is used to stain DNA fragments followed by exposure to UV radiation. This gives bright orange colour to DNA fragments which helps to view separated DNA fragments.

19. The correct sequence to accomplish biotechnological reaction is :

Denaturation of ds DNA
↓
Genomic DNA template
↓
Chemically synthesised oligonucleotides (Primers)
↓
Complementary regions of DNA
↓
Thermostable DNA polymerase (Enzyme polymerase) (from *Thermus aquaticus*)
↓
dNTPs (Nucleotides) provided
↓
In vitro synthesis of copies of DNA of interest

20. Genes encoding resistance to antibiotics are considered useful selectable markers for *E. coli* cloning vector because they help in selecting transformant cell from non-transformant ones.

The genes encoding resistance to antibiotics such as tetracycline, ampicillin, kanamycin or chloramphenicol, etc. are useful selectable markers for *E. coli*. The common *E. coli* cells are not resistant to any of these antibiotics. Plasmid pBR322 has two antibiotic resistance genes – ampicillin resistance (amp^R) and tetracycline resistance (tet^R) which are considered useful for selectable markers.

The presence of restriction sites within the markers tetR and ampR permits an easy selection for cells transformed with the recombinant pBR322. For example, insertion of the DNA fragment into the plasmid using enzyme *Pst*I or *Pvu*I places the DNA insert within the gene amp^R. This makes amp^R nonfunctional. Bacterial cells containing such a recombinant pBR322 will be unable to grow in the presence of ampicillin, but will grow on tetracycline.

21.

Isolation of DNA fragments or gene to be cloned
↓
The purified DNA and vector DNA are cut by the restriction enzyme *Eco*RI.
↓
Insertion of isolated gene in the plasmid vector
↓
Introduction of the recombinant DNA into a suitable cell called host
↓
Selection of transformed host cell, and identification of the clone containing the desired gene/DNA fragment
↓
Multiplication/expression of the introduced gene in the host.

22. (a) Labelled part A represents the enzyme reverse transcriptase. Reverse transcriptase is used to synthesise DNA or complementary DNA by using *m*RNA as template.

(b) The labelled part B represents the separated single-stranded complementary DNA after the RNA-DNA complex is treated with alkaline phosphatase enzyme. The labelled part C is the newly formed double-stranded cDNA.

(c) This diagram represents the synthesis of complementary DNA or cDNA from RNA. With the help of the enzyme reverse transcriptase, an RNA-DNA complex is formed from RNA (usually *m*RNA) where RNA acts as the template. The *m*RNA in the RNA-DNA complex is digested in the presence of alkaline phosphatase enzyme. A cDNA is formed on the separated

single-stranded DNA template with the help of the enzyme DNA polymerase.

23. Differences between rDNA and cDNA are as follows:

	rDNA	cDNA
1.	rDNA stands for recombinant DNA.	cDNA stands for complementary DNA.
2.	It is formed by isolating DNA with desirable genes and then introducing these genes into host.	It is obtained from *m*RNA template by using the enzyme reverse transcriptase.

24. Some genes called selectable markers help in selecting those host cells which contain the vectors and eliminating the non-transformants. β-galactosidase is an alternative selectable marker developed to differentiate recombinants and non-recombinants on the basis of their ability to produce colour in the presence of a chromogenic substance. A recombinant DNA is inserted in the coding sequence of an enzyme β-galactosidase. This causes inactivation of the enzyme which is called insertional inactivation. If the plasmid in the bacterium does not have an insert, the presence of a chromogenic substrate gives blue coloured colonies.

Presence of insert results into insertional inactivation of the β-galactosidase and, therefore, the colonies do not produce any colour, these colonies are marked as recombinant colonies.

β-galactosidase is a preferred selectable marker to antibiotic resistance genes because due to inactivation of antibiotics, selection of recombinants becomes burdensome process as it requires simultaneous plating on two plates having different antibiotics. But by using β-galactosidase as selectable marker, we can select recombinants and non-recombinants on a single plate.

25. A bioreactor is a device in which raw materials are biologically converted into specific products by microbes, plant/animal cells etc. These are used for food processing, fermentation, waste treatment, etc.

Growth conditions that a bioreactor provides for obtaining the desired products are :

(i) Controlled environment for optimum product yield.

(ii) Aseptic fermentation for a number of days and prevention of escape of viable cells.

(iii) Adequate mixing and aeration for optimum growth and production, without damaging the microorganism.

(iv) Easy and dependable temperature control

(v) Facility of sampling.

26. *Taq* DNA polymerase isolated from thermophilic bacterium *Thermus aquaticus* synthesises the DNA region from gene of interest between the primers, using dNTPs (deoxynucleotide triphosphates) and Mg^{2+}. The primers are extended towards each other so that the DNA segment lying between the two primers is copied. It is stable even at high temperatures.

Taq polymerase is heat stable enzyme and is able to withstand high temperature induced denaturation of DNA during PCR. Hence it is preferred in PCR reactions.

27. (a) DNA is a hydrophilic molecule, so it cannot pass into a host cell through cell membrane. The cell membrane consists of lipid bilayers that are generally impermeable to hydrophilic molecules.

(b) DNA is interwined with proteins like histones and RNA. To obtain purified DNA, proteases are added during isolation of DNA which convert proteins into amino acids. The purified DNA finally precipitates out after the addition of chilled ethanol.

(c) In order to link the alien DNA, the vector needs to have very few, preferably single, recognition sites for the commonly used restriction enzymes. Presence of more than one recognition sites within the vector will generate several fragments, which will complicate the gene cloning process.

28. (a) Competent host is essential for transformation with recombinant DNA. Since DNA is a hydrophilic molecule, it cannot pass through membranes, so the bacterial cells must be made capable to take up DNA *i.e.*, made competent. This is done by treating them with a specific concentration of a divalent cations, such as calcium 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 cells 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.

Other methods are:

(i) Microinjection : DNA is inserted through microneedles or micropipettes.

(ii) Electroporation : Electric impulse induce transient pores.

(iii) Gene gun or biolistic : DNA coated with microscopic pellets of gold or tungsten is shot with high velocity into target cells.

(b) Biolistic gun helps in the process of gene transfer into the host cell without using a vector. In biolistic method or gene gun method, tungsten or gold particles, coated with foreign DNA are bombarded into target cells at a very high velocity. This method is suitable for plants, but is also used to insert genes into animal that promote tissue repair into cells (particularly cancer of mouth) near wounds. It has made great impact in the field of vaccine development.

29. PCR (Polymerase Chain Reaction).

The two sets of primers (small chemically synthesised oligonucleotides that are complementary to the regions of DNA) are required in each cycle of polymerase chain reaction. Primers hybridise to target DNA region and allow synthesis of the DNA towards one another whereas DNA polymerase synthesise DNA region between the primers using dNTPs and Mg^{2+}. Three diagnostic applications of PCR are :

(i) Diagnosis of pathogens

(ii) Diagnosis of specific mutations

(iii) Prenatal diagnosis

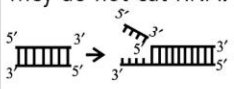
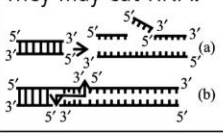
30. (i) *ori* : *ori* is the origin of replication. This is a sequence from where replication starts and any piece of DNA when linked to this sequence can be made to replicate within host cells. It controls the copy number of the linked DNA.

(ii) amp^R : Gene for ampicillin resistance which helps in selecting the transformants.

(iii) *rop* : *rop* codes for the proteins involved in the replication of the plasmid.

31. *Agrobacterium tumefaciens* is a soil-inhabiting bacterium that may invade growing plants at the junction of root and stem, where it can cause a cancerous growth known as a crown gall. *A. tumefaciens* contains Ti plasmid which carries gene for tumour formation for using *Agrobacterium tumefaciens* as a cloning vector researchers deleted the genes which governs auxin and cytokinin production (the oncogene) from T-DNA of Ti plasmid, it is known as disarming. After disarming, this T-DNA is inserted into chromosomes of the host plant where it produces copies of itself.

32. (a) Differences between action of exonucleases and endonucleases are as follows :

	Exonucleases	Endonucleases
(i)	These nucleases cleave base pairs of DNA at their terminal ends.	They cleave DNA at any point except the terminal ends.
(ii)	They act on single strand of DNA or gaps in double stranded DNA.	They cleave one strand or both strands of double stranded DNA.
(iii)	They do not cut RNA. 	They may cut RNA. 

(b) Restriction nucleases act as molecular scissors or chemical scalpels. 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 sugar-phosphate backbones. Each restriction endonuclease recognises a specific palindromic nucleotide sequence in the DNA.

33. Refer to answer 31

34. (a) Enzyme *Eco*RI is named as follows :

The capital letter E comes from the genus *Escherichia*. The letters *co* are derived from the species name *coli*. The letter R is from RYI3 (strain). The Roman number I indicates that it was the first enzyme isolated from the bacterium *E. coli* RYI3.

(b) *Eco*RI is a restriction endonuclease enzyme it recognises 5′-GAATTC-3′ base sequences 3′-CTTAAG-5′ in DNA duplex.

(c) It cut each of the two strands between G and A producing sticky ends.

35. (i) The palindromic sequence in DNA is a sequence of base pairs that reads same on the two strands when orientation of reading is kept the same.

(ii) The palindrome sequence in the given DNA strand is :

GAATTC

CTTAAG

This is the recognition sequence for restriction enzyme *Eco*RI.

(iii) Each restriction endonucleases recognise a specific palindromic nucleotide sequences in the DNA. Restriction enzymes cut the strand of DNA a little away from the centre of the palindrome sites, but between the same two bases on the opposite strands. This leaves single stranded portions at the ends. These are overhanging stretches called sticky ends on each strand. These are named so because they form hydrogen bonds with their complementary cut counterparts. This stickiness of the ends facilitates the action of the enzyme DNA ligase.

36. The cloning vectors are DNA molecules that can carry a foreign DNA segment and replicate inside the host cell. These are plasmids, cosmids, phagemids, yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), transposons and virus.

Cloning vector carry rDNA and they generally have high copy number, they can produce multiple number of required gene. Vectors help in easy linking of foreign DNA and in selection of recombinants from non recombinants.

The entire procedure of gene cloning or recombinant DNA technology may be classified into the following six steps for the convenience in description and on the basis of the chief activity performed.

(i) Production and isolation of the DNA fragments to be cloned.

(ii) Insertion of the isolated gene in a suitable vector to obtain recombinant DNA.

(iii) Introduction of the recombinant DNA into a suitable organism/cell (usually *E.coli*) called host (transformation).

(iv) Selection of the transformed host cells, and identification of the clone containing the desired gene/DNA fragment.

(v) Multiplication/expression of the introduced gene in the host.

37. (a) Desirable DNA sequences are cut by the use of enzyme restriction endonuclease. The restriction enzymes cut the strand of DNA a little away from the centre of the palindromic sites, between the same two bases on the opposite strands, it leaves single stranded portions at the ends. This forms overhanging stretches called sticky ends on each strand. They are called sticky as they form hydrogen bonds with their complementary cut counterparts. The stickiness of the ends facilitates the action of the enzyme DNA ligase.

(b) The three steps involved in each cycle of PCR are :

(i) Denaturation

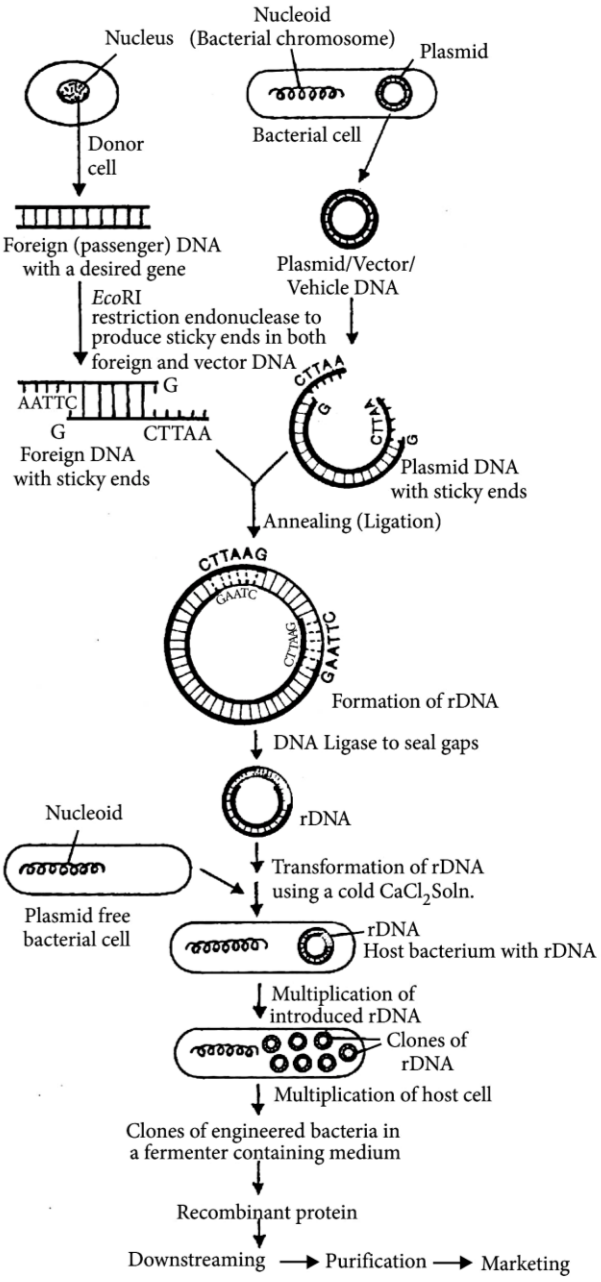
(ii) Annealing

(iii) Extension

PCR is based on the principle that a DNA molecule, when subjected to high temperature, splits into two strands due to denaturation. These single stranded DNA molecules are then converted to original double stranded molecules, in the presence of enzyme DNA polymerase. A double stranded molecule of DNA is duplicated in this way and multiple copies of original DNA sequence can be generated by repeating the process several times. Such repeated amplification is achieved by the use of thermostable DNA polymerase (isolated from *Thermus aquaticus*), which remain active during the high temperature induced denaturation of double stranded DNA.

38. (a) *Refer to answer 36.*

(b) Diagram showing various steps of recombinant DNA technology is given below:



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