CHAPTER – 11

BIOTECHNOLOGY: PRINCIPLE AND PROCESSES

The technique of using life organisms or enzymes from organisms so as to produce products and processes that are useful to humans is called biotechnology.

According to **European Federation of biotechnology** (EFB), Biotechnology is the integration of natural science and organisms, cells, parts thereof, and molecular analogs for products and services.

Principles of Biotechnology

Biotechnology has two main techniques:

Genetic Engineering: Genetic engineering is described as the alteration of an organism's genome (DNA and RNA). It entails the introduction of additional genes into host species in order to improve their function or characteristic, hence altering the host organism's phenotype.

Bioprocess engineering: Sterility is maintained in chemical engineering processes to allow only desirable bacteria to thrive for the production of biotechnological goods such as antibiotics, vaccines, enzymes, and so on.

The technique of genetic engineering includes:

- Creation of recombinant DNA
- Gene cloning
- Gene transfer

The fate of the piece of DNA transferred to alien organism: the DNA transferred into the alien organism may not be able to multiply itself in the progeny cells of the organism but when it gets integrated into the genome of the recipient it may multiply and be inherited along with the host DNA.

This is because the alien piece of DNA has now become a part of a chromosome which has the ability to replicate in a chromosome.

There is a specific DNA sequence called the **'origin of replication'** that is responsible for initiating the replication, so for the multiplication of any alien piece of DNA in an organism, it needs to be a part of a chromosome that has a specific sequence known as **'origin of replication'.**

So alien DNA is linked with the '**origin of replication'** so that this alien piece of DNA can replicate and multiply itself in the host organism, it is also called as **cloning.**

Construction of an artificial rDNA molecule:

- The potential of connecting an antibiotic resistance gene with a native Plasmid of ***Salmonella typhimurium*** led to the creation of the first recombinant DNA.
- **Stanley Cohen and Herbert Boyer** extracted the antibiotic resistance gene by removing a fragment of DNA from a *Salmonella typhimurium* plasmid (autonomously reproducing circular extra-chromosomal DNA). The discovery of molecular scissors'–restriction enzymes–made it feasible to cut DNA at specified spots.
- The cut piece of DNA was linked with the plasmid DNA with the help of DNA ligase that acts on the cut ends of the DNA molecules and then joins their ends, this gives rise to rDNA.
- When this DNA is transferred into *E.coli*, it has the potential to replicate numerous times utilising the new host DNA polymerase enzyme. Cloning of antibiotic resistance gene in *E.coli* refers to the capacity to multiply copies of an antibiotic resistance gene in *E.coli.*

- These "recombinant DNA" (rDNA) molecules are then introduced into host cells, where they can be propagated and multiplied.

ASSERTION AND REASON

Q1. EFB stands for.
(a) Ethiopian Federation for Biotechnology
(b) European Federation for Biotechnology
(c) Ecuador Federation for Biotechnology
(d) None of the above

S1. (b)

Q2. Which of the following are also called as 'molecular scissors'?
(a) DNA polymerases
(b) Restriction endonucleases
(c) RNA polymerases
(d) All of the above

S2. (b)

Tools of Recombinant DNA technology

The major tools that are used in rDNA technology are:

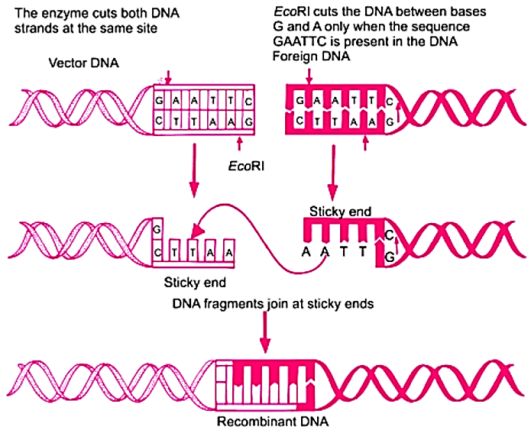
- Enzymes: the major enzymes used in rDNA technology are:
- Molecular scissors: these are the restriction enzymes, that belong to the class **Nucleases.** They are of two types:

(i) **Endonucleases:** they remove nucleotides from somewhere within the DNA, it is very helpful in producing specific cuts in the DNA. The specific base sequence is of six base pairs.

Palindromes: Palindromes are group of letters that form the same words when read both forward and backward. E.g. "MALYALAM".

(ii) **Exonucleases:** they remove nucleotides from the ends of the DNA. E.g. Hind II. Each restriction endonuclease is unique to a palindromic nucleotide sequence in the DNA.

Today, we know more than 900 restriction enzymes that have been isolated from over 230 strains of bacteria each of which recognise different recognition sites.

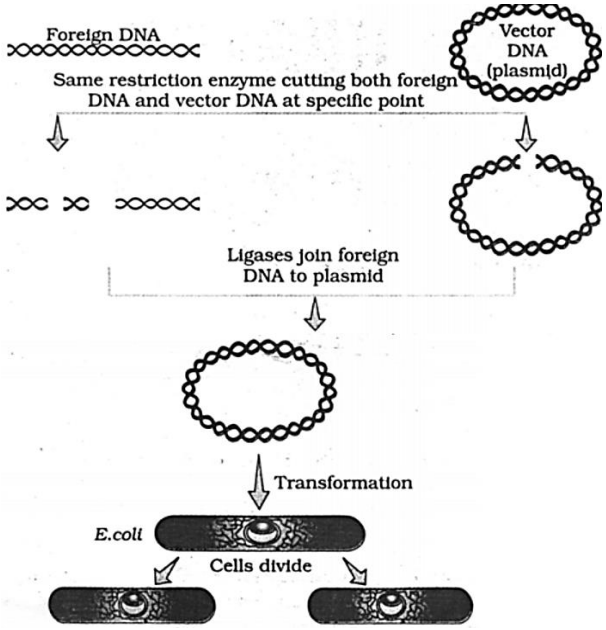


Steps in the formation of DNA by the action of restriction endonuclease enzyme EcoRI.

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. Restriction enzymes cut the strand of DNA a bit away from the palindrome site's centre between the identical two bases on opposing strands with sticky strand. The strands' stickiness facilitates the operation of the enzyme DNA ligase.

Restriction endonucleases are utilised in genetic engineering to create recombinant DNA molecules made up of DNA from diverse sources or genomes.

When the same restriction enzyme is used to cut the DNA, the resulting fragments contain the same type of **Sticky-ends,** the stickiness of the ends facilitates the action of the enzyme DNA ligase.

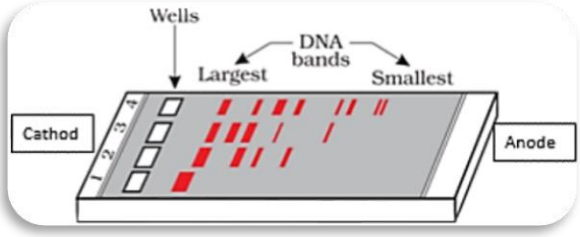


Representation of recombinant DNA technology

Agarose gel Electrophoresis:

- ✓ the cutting of DNA by restriction endonucleases results in the fragments of DNA fragments can be separated by a technique known as gel electrophoresis.
- ✓ DNA is negatively charged and hence it can be separated by making them move towards the anode under an electric field through a matrix.
- ✓ the commonly used Matrix in DNA gel electrophoresis is agarose that is a natural polymer extracted from seaweeds.
- ✓ DNA fragments resolve according to the size through the sieving effect provided by the agarose gel.
- ✓ **the DNA fragments that are separated can be seen after staining the DNA with ethidium Bromide and later by exposing it to ultraviolet radiation**.
- ✓ the separated DNA fragments can be seen as orange colour bands that can be cut out from the gel and purified from the gel, this process is called DNA **elution.**

✓ the DNA fragments are used in constructing the Recombinant DNA by attaching them with cloning vectors.



A typical agarose gel electrophoresis

- Polymerases: These enzymes catalyse the synthesis of DNA molecules from nucleoside diphosphate and are essential for DNA replication, they usually work in groups to create two identical DNA duplexes from a single original DNA duplex, during this process DNA polymerase reads the existing DNA strands to create two news strands that match the existing one.
- Ligases: these enzymes catalyse the joining of two large molecules of DNA by forming a chemical bond.

Brush Up Your Understanding

Q1. Molecular scissors belong to which of the following class of enzymes?
(a) Nucleases (b) Hydrolases
(c) Lyases (d) Ligases

S1. **(a)**

Q2. Which of the following is used to dye the DNA fragments so as to view it under UV radiation?
(a) Methyl orange (b) Ethidium bromide
(c) Indigo (d) Malachite green

S2. **(b)**

- **Vectors: Plasmids and Bacteriophages** are two typical vectors for cloning. They have the ability to multiply within bacterial cells independently of chromosomal DNA regulation. Bacteriophages have very **high copy** numbers of their genome within bacterial cells due to their large frequency per cell.

  For example, *Agrobacterium tumifaciens,* a pathogen of several dicot plants is able to deliver a piece of DNA known as 'T-DNA' to transform normal plant cells into a tumor and direct these tumor cells to produce the chemicals required by the pathogen. The tumor inducing (Ti) plasmid of *Agrobacterium tumifaciens* has now been modified into a cloning vector which is no more pathogenic to the plants but is still able to use the mechanisms to deliver genes of our interest into a variety of plants

  Similarly, retroviruses in animals have the ability to transform normal cells into cancerous cells. They have also been disarmed and are now used to deliver desirable genes into animal cells.

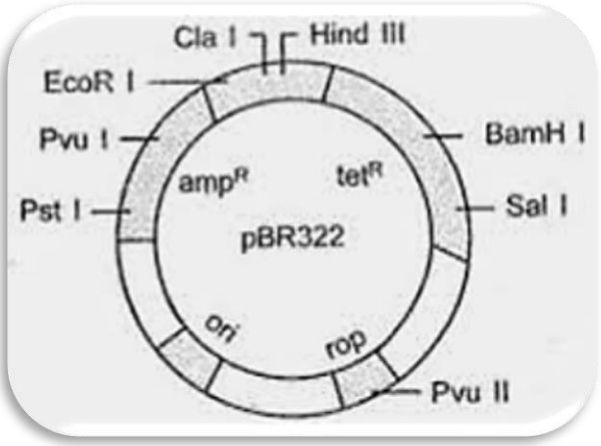
  The main features a vector should have for gene cloning are:

(i) Origin of replication (*ori):* When any fragment of DNA is joined to this sequence, it may be made to reproduce within the host cells. This segment is in charge of regulating the copy number of the connected DNA.

(ii) Selectable marker: this assists in recognising and removing non-transformants while selectively allowing the development of transformants. **Transformation is the process of inserting a fragment of DNA into a host bacteria. In general, genes encoding antibiotic resistance, such as ampicillin, chloramphenicol, tetracycline, or kanamycin, are thought to be helpful selection markers for *E. coli.***

(iii) Cloning sites: the vector must have a single recognition site for the generally used restriction enzymes for joining the foreign DNA, as multiple recognition sites inside the vector may yield several fragments, that may make the gene cloning process tricky. The foreign DNA is ligated at a restriction point found in one of the two antibiotic resistance genes.

e.g. *E.coli* cloning vector pBR322 shown in the diagram.



***E.coli* cloning vector pBR322**

Insertional Inactivation: the cloned DNA fragment disrupts the coding sequence of a gene, this is called insertional inactivation, the method is very important in screening recombinants.

e.g. Blue white selection:

(i) The insertional inactivation of the lac Z gene contained on the vector is the basis for this approach.

(ii) The lac Z gene encodes the beta-galactosidase enzyme, which may convert a chromogenic substrate into a blue product.

(iii) **If this lac Z gene is inactivated by inserting a target DNA fragment into it, the formation of blue colonies is stopped, and white colonies result.**

(iv) This allows us to distinguish between recombinant (white) and non-recombinant (blue) colonies.

- **Host organisms:** these are the bacterial cells that take up the recombinant DNA, as DNA is hydrophilic, it cannot pass through the cell membrane of bacteria, thus the bacterial cells have to be made competent to take up the DNA. This is done as follows:

(a) **A simple chemical treatment with divalent calcium ions boosts the efficacy** of host cells to take up the rDNA plasmids (through cell wall pores).

(b) rDNA may also be turned into host cells by incubating both on ice, then temporarily placing them at 42 degree C **(Heat Shock),** and then returning to ice. This allows the bacteria to consume the recombinant DNA.

(c) Using a glass micropipette, rDNA is directly injected into the nucleus of cells in the **Microinjection technique.**

(d) **Biolistics / Gene gun approach,** which has been created to transfer rDNA into plant cells primarily by the use of a Gene / Particle gun. In this procedure, minute gold/tungsten particles are coated with the desired DNA and battered onto cells.

(e) The last technique employs **"Disarmed Pathogen" Vectors (*Agrobacterium tumefaciens*)**, which, once infected, transport the recombinant DNA into the host.

Brush Up Your Understanding

Q1. The features a vector should have for it to be perfect for gene cloning is.

(a) Cloning sites (b) Selectable marker
(c) Ori (d) All of the above

S1. **(d)**

Q2. To make the bacterial cells competent, they are given heat shock at a temperature.

(a) 41 degree (b) 42 degree
(c) 43 degree (d) 44 degree

S2. **(b)**

How does the Recombinant DNA technology works?

Various steps in recombinant DNA technology are as follows:

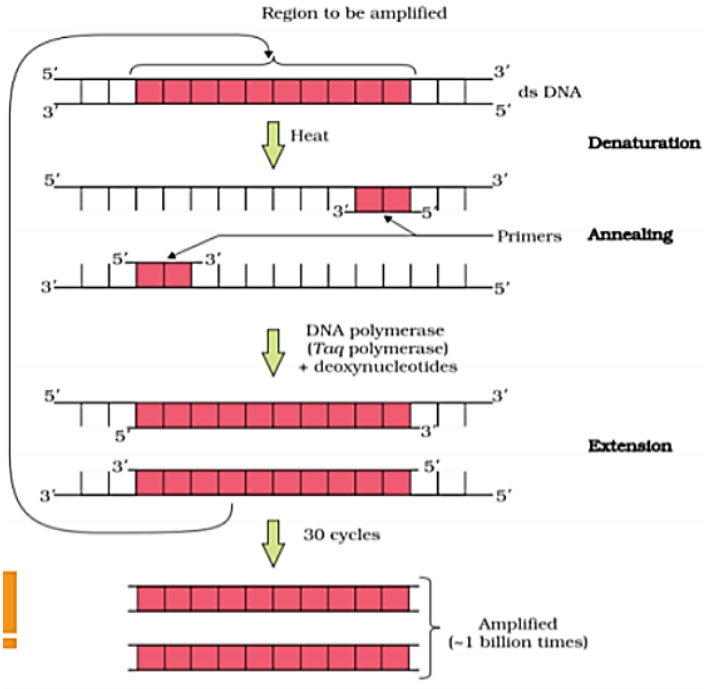
- ✓ Isolation of DNA
- ✓ Fragmentation of DNA by restriction endonuclease
- ✓ Isolation of desired DNA fragment
- ✓ Ligation of DNA fragment into the vector
- ✓ Transforming the recombinant DNA into the host
- ✓ Culturing the host cells in a medium at large scale
- ✓ Extraction of the desired product:

Isolation of Genetic material: Enzymes such as lysozyme, cellulase, and chitinase are used to separate genetic material from other macromolecules (fungus). Spooling can be used to remove DNA that has separated. RNA can be removed by using ribonuclease, whereas proteins may be removed by using protease.

Cutting of DNA at specific location: To access the course of a restriction enzyme digestion, restriction enzyme and Agarose gel electrophoresis are used. After cutting the source and vector DNA with a particular restriction enzyme to remove the 'gene of interest' from the source DNA.

Amplification of gene of interest with PCR: here the gene of interest is amplified and multiple copies are made with help of primers, polymerases and a set of primers.

Insertion of Foreign DNA into the host cell: there are several methods of introducing the recombinant DNA into recipient cells, the recipient cells after making them competent to receive, take up DNA present in a surrounding, thus if a recombinant DNA bearing piece for resistance to an antibiotic (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 and the untransformants will die.



Polymerase chain Reaction

Note: a thermostable DNA polymerase (isolated from a bacterium, *Thermus aquaticus),* which remains active during the high temperature induced denaturation of double stranded DNA.

After having cloned the gene of interest and having optimised the conditions to induce the expression of the target protein, one has to consider producing it on a large scale.

Small volume cultures cannot yield appreciable quantities of products. To produce in large quantities, the development of bioreactors, where large volumes (100-1000 litres) of culture can be processed, was required. Thus, 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 common types of bioreactors used 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.

Brush Up Your Understanding

Q1. In DNA purification protocol, the DNA ultimately precipitates out during the procedure by the addition of pure.
(a) Iso propyl alcohol (b) Ethanol
(c) Methyl alcohol (d) None of the above

S1. (b)

Q2. In PCR technique, annealing is the.
(a) 1st step of the protocol
(b) 2nd step of the protocol
(c) 3rd step of the protocol
(d) 4th step of the protocol

S2. (b)

Down Stream Processing

After completion of the biosynthetic stage, the product has to be subjected through a series of processes before it is ready for marketing as a finished product. The processes include separation and purification, which are collectively referred to as downstream processing. The product has to be formulated with suitable preservatives.

SUMMARY

The use of biology to develop technologies and products for the welfare of human beings is known as biotechnology. The two core techniques that give rise to modern biotechnology are genetic engineering and bioprocess engineering. Genetic engineering allows the isolation and introduction of only the desired genes into the organism without introducing the undesirable genes, main tools of genetic engineering are restriction enzymes, vectors, host.

The basic steps in genetic modification of an organism are the identification of desired DNA fragment, introduction of desired DNA fragment into suitable host, maintaining foreign DNA in the host and its transfer to the progeny.

Agarose gel electrophoresis is the technique in which the DNA fragments obtained through restriction are separated.

To produce large quantities of recombinant protein, large vessels known as bioreactors are used. A bioreactor provides the optimal conditions for achieving the desired product by providing optimum growth conditions (temperature, pH, substrate, salts, vitamins, oxygen).

The processes and methods involved in the separation and purification of the desired product are called as downstream processing. In case of drugs, the product needs to be suitably formulated and drug tested before being made available commercially.

IMPORTANT POINTERS

The techniques of genetic engineering which include creation of recombinant DNA, use of gene cloning and gene transfer.

In the year 1963, the two enzymes responsible for restricting the growth of bacteriophage in *Escherichia coli* were isolated called as restriction endonucleases.

Today we know more than 900 restriction enzymes that have been isolated from over 230 strains of bacteria.
Restriction enzymes belong to a larger class of enzymes called nucleases.

The separated DNA fragments on agarose gel electrophoresis can be viewed under UV light after staining it with an intercalating agent called ethidium bromide that absorbs light in the range of 260-330 nm.

Microinjection, biolistics and 'disarmed pathogen' vectors are used to introduce alien DNA in the host cell.
The most commonly used bioreactors in recombinant DNA technology are are of stirring type.

MULTIPLE CHOICE QUESTIONS

Q1. Which of the following are part of biotechnology?
(a) In-vitro fertilisation leading to test tube baby
(b) Synthesising a gene
(c) Developing a DNA vaccine
(d) All of the above

Q2. Which of the following is the definition given by the European Federation of biotechnology that encompasses both traditional views and modern molecular biotechnology?
(a) the innovation of natural science and organism's cells, parts thereof, and molecular analogues for products and services
(b) the integration of natural science and organism's cells, parts thereof, and molecular analogue for products and services
(c) the inactivation of natural science and organisms cells, parts thereof, and molecular analogue for products and services
(d) the integration of organisms cells, parts thereof, and molecular analogue for products and services

Q3. Which of the following techniques enables the birth of modern biotechnology?
(a) Plant breeding
(b) Genetic engineering
(c) Bioprocess engineering
(d) Both (b) and (c)

Q4. What is genetic engineering?
(a) it is a technique to alter the chemistry of genetic material DNA
(b) it is a technique to alter the chemistry of genetic material RNA
(c) it is a technique to alter the chemistry of genetic material DNA and RNA
(d) it is a technique to alter the chemistry of genetic material protein

Q5. Genetic engineering includes.
(a) Creation of recombinant DNA
(b) Gene cloning
(c) Gene transfer
(d) All of the above

Q6. Which of the following is responsible for initiating replication in a chromosome?
(a) Promoter
(b) Origin of replication
(c) Plasmid
(d) None of the above

Q7. What is cloning?
(a) making single identical copy of any template DNA
(b) making multiple identical copies of any template DNA
(c) making multiple identical copies of any protein
(d) making multiple identical copies of chromosome

Q8. What is gene transfer?
(a) the introduction of new protein in an existing organism cell
(b) the introduction of new DNA in an existing organism cell
(c) the introduction of new RNA in an existing organism cell
(d) the introduction of chromosome in an existing organism cell

Q9. What is a plasmid?
(a) it is an autonomously replicating circular extrachromosomal DNA
(b) it is an autonomously replicating linear extrachromosomal DNA
(c) it is an autonomously replicating circular chromosomal DNA
(d) it is an autonomously replicating circular extrachromosomal RNA

Q10. Who among the following accomplished the construction of the first component DNA in 1972?
(a) Von Bear
(b) Alexander Fleming
(c) Stanley Cohen and Herbert Boyer
(d) Ernst Heckel

Q11. Which of the following is also referred to as molecular scissors?
(a) Polymerases (b) Restriction enzymes
(c) Transcriptase (d) None of the above

Q12. What is the use of vector in biotechnology?
(a) They help in the transfer of piece of RNA attached to it
(b) They help in the transfer of piece of protein attached to it
(c) They help in the transfer of piece of DNA attached to it
(d) They help in the transfer of piece of chromosome attached to it

Q13. Which of the following enzyme at once cut DNA molecules and join their ends?
(a) DNA polymerase
(b) Restriction enzymes
(c) DNA ligase
(d) Reverse transcriptase

Q14. Which of the following enzyme always cut DNA molecules at a particular point by recognising a specific sequence of 6 base pairs?
(a) Bam H 1 (b) Eco R 1
(c) Hind II (d) Hind III

Q15. How many restriction enzymes have been discovered till now?
(a) More than 1000 (b) More than 900
(c) More than 800 (d) More 700

Q16. Which of the following is a source of Eco R 1?
(a) *Escherichia coli* RY11 (b) *Escherichia coli* RY12
(c) *Escherichia coli* RY13 (d) *Escherichia coli* RY14

Q17. Which among the following is the function of exonucleases?
(a) the remove nucleotides from the middle of the DNA
(b) the remove nucleotides from the ends of the DNA
(c) the remove nucleotides from the template DNA
(d) the remove nucleotides from newly processed DNA

Q18. Which of the following is correct about restriction endonucleases?
(a) they recognise a specific nucleotide sequence in the DNA
(b) they recognise a specific palindromic sequence in the DNA
(c) they recognise a specific palindromic nucleoside sequence in the DNA
(d) they recognise a specific palindromic nucleotide sequences in the DNA

Q19. What is the charge on the DNA molecule?
(a) Positive charge
(b) Neutral
(c) Negative charge
(d) None of the above

Q20. Name the matrix that is used to separate DNA fragments using gel electrophoresis.
(a) Cellulose (b) Agar
(c) Agarose (d) Starch

Q21. Which of the following compound is used to stain the separated DNA fragments to view them under the UV radiation?
(a) Methyl orange (b) Ethidium bromide
(c) Methyl red (d) Naphthol green B

Q22. Which of the following is responsible for controlling the copy number of the linked DNA during replication?
(a) Selectable marker
(b) Origin of replication
(c) Cloning sites
(d) Promoter

Q23. What is transformation?
(a) it is a procedure through which a piece of RNA is introduced into a host bacterium
(b) it is a procedure through which a piece of protein is introduced into a host bacterium
(c) it is a procedure through which a piece of chromosome is introduced into a host bacterium
(d) it is a procedure through which a piece of DNA is introduced into a host bacterium

Q24. Which of the following diavalent cation is used to increase the efficiency of DNA so that it easily enters the bacterium through the pores present in its walls?
(a) Magnesium (b) Barium
(c) Calcium (d) All of the above

Q25. Which of the following metal is used for DNA in the host cell through gene gun technology?
(i) Silver (ii) Gold
(iii) Tungsten (iv) Both (b) and (c)

Q26. Which of the following enzyme is used to treat the bacterial cells for the isolation of DNA?
(a) Lysozyme (b) Cellulase
(c) Chitinase (d) All of the above

Q27. Which of the following can be used to remove RNA during DNA isolation procedure?
(a) Proteases
(b) Ribonucleases
(c) DNAses
(d) Restriction endonucleases

Q28. Which of the following is used to precipitate purified DNA during the procedure of DNA isolation?
(a) Chilled methanol (b) Chilled water
(c) Chilled ethanol (d) Chilled buffer

Q29. Which of the following is the correct form of PCR?
(a) Polymer Chain Reaction
(b) Pole Chain Reaction
(c) Prime Chain Reaction
(d) Polymerase Chain Reaction

Q30. What is a recombinant protein?
(a) it is a protein encoding DNA that is expressed in a heterologous host
(b) it is a protein encoding gene that is expressed in a heterologous host
(c) it is a protein encoding RNA that is expressed in a heterologous host
(d) it is a protein encoding ribosome that is expressed in a heterologous host

Q31. In gel electrophoresis, DNA fragments are separated on the basis of.
(a) Charge (b) Size
(c) Weight (d) All of the above

Q32. Which of the following helps in the selection of transformed cells on a Petri dish?
(a) Antibiotic resistance gene
(b) Recognition sites
(c) Cloning sites
(d) Ori C

Q33. Eco R1 is a restriction enzyme. In the name, what does 'co' stands for?
(a) Colon (b) Coli
(c) Coffee (d) Coenzyme

Q34. Which of the following is the source of restriction endonucleases?
(a) Fungal cells (b) Bacterial cells
(c) Plant cells (d) Virus

Q35. After running gel electrophoresis, the extraction of DNA fragments from agarose gel is called as.
(a) Cutting (b) Elution
(c) Separation (d) Ligation

Q36. Which of the following is a protein digesting enzyme?
(a) Chitinase (b) Cellulase
(c) Protease (d) Lipase

Q37. Which of the following is correct about plasmid?
(a) It is bacteria
(b) It is extra chromosomal DNA
(c) It is a plant
(d) It is virus

Q38. What are bacteriophages?
(a) They are fungus that infect bacteria
(b) They are virus that infect bacteria
(c) They are protozoans that infect bacteria
(d) They are helminths that bacteria

Q39. What are basic steps of genetically modifying an organism?
(a) identification of DNA with desirable genes
(b) introduction of the identified DNA into the host
(c) maintenance of introduce DNA in the host and transfer the DNA to its progeny
(d) All of the above

Q40. Which of the following cannot be included under biotechnology?
(a) Development of COVID vaccine
(b) Test tube baby technique
(c) Setting of curd
(d) Treating defective gene

Q41. Which of the following is the correct full form of EFB?
(a) European Federation of Biotechnology
(b) Eastern Federation of Biotechnology
(c) Ethopian Federation of Biotechnology
(d) European Federation of Biology

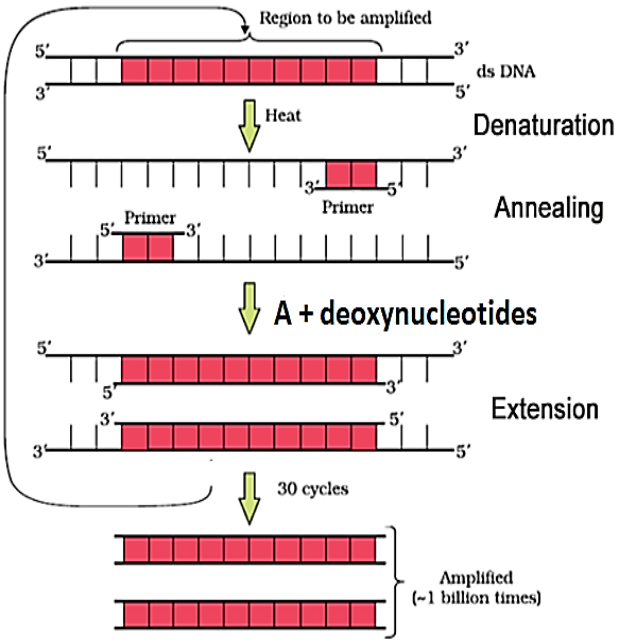
Q42. What is the meaning of annealing in PCR technique?
(a) It is the joining of RNA primers to the template DNA
(b) It is the joining of DNA primers to the template DNA
(c) It is the joining of protein to the template DNA
(d) It is the joining of DNA primers RNA

Q43. *Taq* polymerase is isolated from.
(a) Thermus aquaticaus
(b) Aspergillus niger
(c) Clostridium butylicum
(d) None of the above

Q44. What is the number of steps in each cycle of PCR?
(a) 1 (b) 2
(c) 3 (d) 4

Q45. What is denaturation of DNA?
(a) It is the separation of ss DNA into strands
(b) It is the separation of ds RNA into single strands
(c) It is the separation of ss RNA into single strands
(d) It is the separation of ds DNA into single strands

Q46. Look at the diagram given below and name 'A'.



(a) DNA ligae
(b) Reverse transcriptase
(c) Proteases
(d) *Taq* polymerase

Q47. What are the optimum growth conditions for large scale production of cultures in a bioreactor?
(a) Temprature, oxygen (b) pH, vitamins
(c) Substrate and salts (d) All of the above

Q48. What are palindromic sequences?
(a) A sequence on ds DNA or RNA which if read in one direction is identical to sequence in the same direction on the complementary strand also.
(b) A sequence on only ds DNA which if read in one direction is identical to sequence in the same direction on the complementary strand also.
(c) A sequence on ds RNA which if read in one direction is identical to sequence in the same direction on the complementary strand also.
(d) A sequence on protein if read in one direction is identical to sequence in the same direction on the complementary strand also.

Q49. Which of the following is not a restriction enzyme?
(a) Bam H1 (b) Hind III
(c) Eco RI (d) Lipase

Q50. Sticky ends of DNA fragments during recombinant DNA technology can be joined by.
(a) DNA polymerase (b) RNA polymerase
(c) DNA ligase (d) *Taq* polymerase

ASSERTION AND REASON

Direction: in the following questions, a statement of assertion (A) is followed by a statement of reason (R). Choose the correct option among a, b, c and d.

Q1. **Assertion (A):** DNA ligase plays an important role in recombinant DNA technology.
Reason (R): The linking of antibiotic resistance gene with plasmid vector became possible by enzyme DNA ligase.
(a) Both assertion (A) and reason (R) are true and reason (R) is the correct explanation of assertion (A)
(b) Both assertion (A) and reason (R) are true but reason (R) is not the correct explanation of assertion (A)
(c) Assertion (A) is true but reason(R) is false
(d) Assertion (A) is false but reason(R) is true

Q2. **Assertion (A):** Restriction enzymes belong to a larger classes of enzymes called nucleases.
Reason (R): Each restriction enzyme recognises a specific palindromic sequence in the DNA.
(a) Both assertion (A) and reason (R) are true and reason (R) is the correct explanation of assertion (A)
(b) Both assertion (A) and reason (R) are true but reason (R) is not the correct explanation of assertion (A)
(c) Assertion (A) is true but reason(R) is false
(d) Assertion (A) is false but reason(R) is true

Q3. **Assertion (A):** During the gel electrophoresis, the fragments of DNA move towards the anode.
Reason (R): DNA fragments are negatively charged molecules.
(a) Both assertion (A) and reason (R) are true and reason (R) is the correct explanation of assertion (A)
(b) Both assertion (A) and reason (R) are true but reason (R) is not the correct explanation of assertion (A)
(c) Assertion (A) is true but reason(R) is false
(d) Assertion (A) is false but reason(R) is true

Q4. **Assertion(A):** The selection of recombinants due to the inactivation of antibiotics is a cumbersome procedure.
Reason (R): It requires simultaneous plating on two plates having different antibiotic's.
(a) Both assertion (A) and reason (R) are true and reason (R) is the correct explanation of assertion (A)
(b) Both assertion (A) and reason (R) are true but reason (R) is not the correct explanation of assertion (A)
(c) Assertion (A) is true but reason(R) is false
(d) Assertion (A) is false but reason(R) is true

TRUE AND FALSE

Q1. DNA is a hydrphobic molecule.

Q2. In order to make the bacterial cells competent, they are treated with a specific concentration of a divalent cation, such as calcium, which increases the efficiency with which DNA enters the bacterium through pores in its cell wall.

Q3. In micro-injection, recombinant DNA is directly injected into the cytoplasm of an animal cell.

Q4. Biotechnology deals with large scale production and marketing of products and processes using live organisms, cells or enzymes.

PRACTICE QUESTIONS

Q1. Which of the following tools of recombinant DNA technology is incorrectly paired with its use?
(a) restriction enzyme - Production of RFLPs
(b) DNA ligase-that cuts DNA, creating the sticky ends
(c) DNA polymerase - used in a polymerase chain reaction to amplify section of DNA
(d) reverse transcriptase - production of cDNA from mRNA

Q2. In r-DNA technology or genetic engineering elution means.
(a) Remove the DNA from centrifuge tube after centrifugation
(b) The separated band of DNA are cut out from the gel and extracted from the gel piece
(c) Separation of the recombinant protein from recombinant cell
(d) Insertion of recombinant DNA into the host cell

Q3. If a recombinant DNA bearing gene for ampicillin resistance if transferred into *E.Coli* cells and the host cells are spread on agar plates containing ampicillin, then.
(a) both transformed and untransformed recipient cells will die
(b) both transformed and untrasformed recipient cell will be grow
(c) tranformed recipient cells will grow and untransformed recipient cells will die
(d) transformed recipient cells will die and untransformed recipient cells will grow

Q4. The technique that serves the purpose of early diagnosis of disease or pathogen.
(a) Recombinant DNA technology
(b) Polymerase chain reaction
(c) Enzyme linked immuno sorbent assay
(d) All the above

Q5. To denature the DNA template in PCR it is heated to.
(a) 70°C (b) 54°C
(c) 80°C (d) 94°C

Q6. 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

Q7. If the bacterium does not have any insert, then in the presence of chromogenic substrate, it gives.
(a) Red coloured colonies
(b) Colourless colonies
(c) Blue colonies
(d) Green colonies

Q8. Insertional inactivation results into inactivation of which enzyme?
(a) Transacetylase (b) Permease
(c) *Taq* polymerase (d) β-galactosidase

Q9. The sequence which is responsible for controlling the copy number of the linked DNA is.
(a) Coding sequence
(b) Promoter sequence
(c) Terminator sequence
(d) Ori

Q10. If any protein encoding gene is expressed in a hetero logous host then protein is known as.
(a) Recombinant gene
(b) Recombinant protein
(c) Selectable marker
(d) Homogenous protein

Q11. Group of letters that form the same words when read both forward and backward is called.
(a) Palindrome (b) Same words
(c) Opposite words (d) None of the above

Q12. The enzymes, which remove nucleotides from the ends of the DNA are.
(a) Exonuclease (b) Endonuclease
(c) Cellulase (d) Hydrolase

Q13. When the isolation of genetic material is done the RNA can be removed by treatment with.
(a) Protease
(b) Chitinase
(c) Ribonuclease
(d) Deoxyribonuclease

Q14. 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

Q15. Which one of the following is must in Genetic engineering?
(a) Restriction endonuclease + DNA ligase + polymerases
(b) Restriction exonuclease + DNA polymerase
(c) Alkaline phosphate + DNA Ligase
(d) RNA polymerase + DNA polymerase

Q16. In the vector pBR322 there is.
(a) One selectable marker
(b) Two selectable markers
(c) Three selectable markers
(d) None of the above

Q17. The enzymes responsible for restricting the growth of bacteriophage in *E-coli* were isolated in 1963, these enzyme are.
(a) DNA ligases
(b) Alkaline phosphatases
(c) DNA polymerases
(d) Restriction endonuclease

Q18. During the process of gene amplification using PCR, if very high temperature is not maintained in the beginning, then which of the following steps of PCR will be affected first?
(a) Annealing (b) Extension
(c) Denaturation (d) Ligation

Q19. DNA strands on a gel stained with ethidium bromide when viewed under UV radiation, appear as.
(a) Yellow bands (b) Bright orange bands
(c) Dark red bands (d) Bright blue bands

Q20. Which of the following is a correct sequence of steps in a PCR (Polymerase Chain Reaction)?
(a) Denaturation, Annealing, Extension
(b) Denaturation, Extension, Annealing
(c) Extension, Denaturation, Annealing
(d) Annealing, Denaturation, Extension

Q21. The laws and rules to prevent unauthorised exploitation of bio-resources are termed as.
(a) Biopatenting (b) Bioethics
(c) Bioengineering (d) Biopiracy

Q22. In Recombinant DNA technology antibiotics are used.
(a) to keep medium bacteria-free
(b) to detect alien DNA
(c) to impart disease-resistance to the host plant
(d) as selectable markers

Q23. In a mixture, DNA fragments are separated by.
(a) Bioprocess engineering
(b) Restriction digestion
(c) Electrophoresis
(d) Polymerase chain reaction

Q24. Identify the wrong statement with regard to Restriction Enzymes.
(a) Sticky ends can be joined by using DNA ligases.
(b) Each restriction enzyme functions by inspecting the length of a DNA sequence.
(c) They cut the strand of DNA at palindromic sites.
(d) They are useful in genetic engineering.

Q25. 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

Q26. A gene whose expression helps to identify transformed cell is known as.
(a) Vector (b) Plasmid
(c) Structural gene (d) Selectable marker

Q27. Which of the following is a restriction endonuclease?
(a) Hind II (b) Protease
(c) DNase I (d) RNase

Q28. Which vector can clone only a small fragment of DNA?
(a) Bacterial artificial chromosome
(b) Yeast artificial chromosome
(c) Plasmid
(d) Cosmid

Q29. Biolistics (gene-gun) is suitable for.
(a) Constructing recombinant DNA by joining with vectors
(b) DNA finger printing
(c) Disarming pathogen vectors
(d) Transformation of plants cells

Q30. For transformation, micro-particles coated with DNA to be bombarded with gene gun are made up of.
(a) Silicon or Platinum (b) Gold or Tungsten
(c) Silver or platinum (d) Platinum or zinc

ASSERTION AND REASON

Direction: in the following questions, a statement of assertion (A) is followed by a statement of reason (R). Choose the correct option among a, b, c and d.

Q1. **Assertion (A):** Ethidium bromide helps in visualising DNA in UV light only.
Reason (R): The dye absorbs light in the range of 260-330nm
(a) Both assertion (A) and reason (R) are true and reason (R) is the correct explanation of assertion (A)
(b) Both assertion (A) and reason (R) are true but reason (R) is not the correct explanation of assertion (A)
(c) Assertion (A) is true but reason(R) is false
(d) Assertion (A) is false but reason(R) is true

Q2. **Assertion (A):** In a chromosome there is a specific DNA sequence called the origin of replication, which is responsible for initiating replication.
Reason (R): 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.*
(a) Both assertion (A) and reason (R) are true and reason (R) is the correct explanation of assertion (A)
(b) Both assertion (A) and reason (R) are true but reason (R) is not the correct explanation of assertion (A)
(c) Assertion (A) is true but reason(R) is false
(d) Assertion (A) is false but reason(R) is true

Q3. **Assertion (A):** Endonucleases remove nucleotides from the ends of the DNA.
Reason (R): Each restriction endonuclease functions by 'inspecting' the length of a DNA sequence.
(a) Both assertion (A) and reason (R) are true and reason (R) is the correct explanation of assertion (A)
(b) Both assertion (A) and reason (R) are true but reason (R) is not the correct explanation of assertion (A)
(c) Assertion (A) is true but reason(R) is false
(d) Assertion (A) is false but reason(R) is true

Q4. **Assertion (A):** Transformation is a procedure through which a piece of DNA is introduced in a host bacterium.
Reason (R): Normally, the genes encoding resistance to antibiotics such as ampicillin, chloramphenicol, tetracycline or kanamycin, etc., are considered useful selectable markers for *E. coli.*
(a) Both assertion (A) and reason (R) are true and reason (R) is the correct explanation of assertion (A)
(b) Both assertion (A) and reason (R) are true but reason (R) is not the correct explanation of assertion (A)
(c) Assertion (A) is true but reason(R) is false
(d) Assertion (A) is false but reason(R) is true

SOLUTIONS MULTIPLE CHOICE

S1. **(d)** Biotechnology deals with techniques of using live organisms and enzymes from organisms to produce products and processes are useful to humans

S2. **(b)** many definitions of biotechnology have been proposed but this definition encompasses both traditional view and the modern molecular biotechnology view also

S3. **(d)** Genetic engineering and Biotechnology engineering are the basis of the origin of modern biotechnology

S4. **(c)** Genetic engineering alters the genetic material DNA or RNA and then it is introduced into the host organism so as to change the phenotype of the host organism

S5. **(d)** Genetic engineering allows us to isolate and introduce only a set of desirable genes without introducing the undesirable genes into the target organism

S6. **(b)** Origin of replication is a specific DNA sequence on the chromosome that is responsible for initiating replication

S7. **(b)** Cloning is done when an alien DNA is linked with the origin of replication so that this is alien piece of DNA can replicate and multiply itself in the host organism

S8. **(b)** Gene transfer is an important aspect of genetic engineering

S9. **(a)** A plasmid was used for the construction of the first recombinant DNA by combining it with a gene encoding antibiotic resistance

S10. **(c)** Stanley Cohen and Herbert Boyer accomplished the construction of first recombinant DNA by isolating the antibiotic resistance gene by cutting out a piece of DNA from a plasmid which was responsible for conferring antibiotic resistance

S11. **(c)** The discovery of restriction enzyme made the cutting of DNA at specific location very easy

S12. **(c)** Just as a mosquito acts as an insect vector to transfer the malarial parasite into the human body in the same way vectors like plasmid in biotechnology help in the transfer of a piece of DNA attached to it

S13. **(c)** Linking of any antibiotic resistance gene with the plasmid vector becomes easy with the help of enzyme DNA ligase that has a property to join cut ends of DNA

S14. **(c)** Hind II was the first restriction endonuclease to be discovered and it always cuts DNA molecules a particular point by recognising 6 place face sequence

S15. **(b)** till now more than 900 restriction enzymes have been isolated from over 230 strains of bacteria each of which recognise different recognition sequences

S16. **(c)** Eco R 1 come Sfrom *Escherichia coli* RY11

S17. **(b)** exonucleases belong to the class of enzyme nucleases and remove nucleotides from the ends of the DNA

S18. **(d)** restriction endonucleases are very specific in their function, once it finds it specific points in the sequence it binds to the DNA and cut each of the two strands of the double helix in the sugar phosphate backbone

S19. **(c)** the charge on DNA is negative, this property is widely used to separate DNA fragments using gel electrophoresis technique

S20. **(c)** agarose is a natural polymer extracted from seaweeds

S21. **(b)** after staining the separated DNA fragments with ethidium bromide, one can see orange colour bands of DNA under UV light

S22. **(b)** origin of replication is a sequence from where replication starts, any piece of DNA when linked to the sequence can be made to replicate within the host cell, it also controls the copy number of the linked-to DNA

S23. **(d)** transformation is a procedure of introducing a novel piece of DNA inside the host cell

S24. **(c)** Diavalent cation like calcium increases the porosity of the bacterial wall and introduces the r-DNA into the bacterial cell

S25. **(d)** In gene gun, DNA particles are coated in the gold or tungsten and then injected directly in the host body.

S26. **(d)** lysozyme is obtained from bacterial cells chitinase from fungal cells and cellulase from plants

S27. **(b)** DNA is interwined with protein, histones. RNA is also present that can be removed with the help of enzyme ribonuclease

S28. **(c)** chilled ethanol easily precipitates the purified DNA out of the test tube

S29. **(d)** polymerase chain reaction is a reaction in which multiple copies of a gene of interest is synthesized *in vitro* using two sets of primers and the enzyme DNA polymerase

S30. **(b)** the desired protein can be extracted from the cloned genes and can be used for any purpose

S31. **(b)** in gel electrophoresis the DNA fragments resolve according to the size due to the sieving effect provided by the agrose gel

S32. **(a)** antibiotic resistance genes act as a marker on the vector that helps in selection of the transformed cells

S33. **(b)** Eco R 1 the letter R is derived from the name of the strain and 'co' stands for *coli*

S34. **(b)** in the 1963, two enzymes responsible for restricting the growth of bacteriophages in *E.coli* were isolated. Later more research was made and were found useful, so they were commercially isolated from bacterial cells for research purposes.

S35. **(b)** the separated bands of DNA are cut out from the agarose gel and extracted from the gel piece, this step is called as elution

S36. (c) protein can be easily removed from DNA by treating it with an enzyme called proteases

S37. (b) Plasmid is autonomously replicating unit present in bacterial cells

S38. (b) Bacterial cells can also be infected by pathogens, like virus as they infect humans and plants, such virus is called bacteriophages

S39. (d) All the above 3 are the 3 basic steps of genetically modifying an organism.

S40. (c) biotechnology is a restricted sense today it is used to refer to such processes which use genetically modified organisms to achieve the same on a larger scale

S41. (a) the European Federation of biotechnology has given a definition of biotechnology that encompasses both traditional view and modern molecular biotechnology

S42. (b) Annealing during PCR is achieved when the temperature is lowered in the machine as per the set protocol.

S43. (a) *taq* polymerase is a thermostable DNA polymerase that is isolated from the bacterium *Thermus aquaticus* and remains active during the high temperature induced denaturation of double stranded DNA

S44. (c) Each cycle of PCR has three steps denaturation, primer annealing and extension of primers

S45. (d) denaturation is the first step of PCR, in this the double stranded DNA opens and converts into single strand so that the primers can anneal

S46. (d) *Taq* polymerase helps in the extension of the primers using deoxynucleotides.

S47. (d) a bioreactor is used to produce large volumes of cultures to obtain the desired protein for the desired product

S48. (a) palindromic sequences are very important in biotechnology, the restriction enzymes cut the strand of DNA a little away from the centre of the palindromic site but between the same two bases on the opposite strands

S49. (d)

S50. (c) DNA ligases effectively joins the sticky ends of DNA during r-DNA technology.

ASSERTION AND REASON

S1. (a)

S2. (b)

S3. (a)

S4. (a)

TRUE AND FALSE

S1. (False) DNA is a hydrophilic molecule.

S2. (True)

S3. (False) In micro-injection, recombinant DNA is directly injected into the nucleus of an animal cell.

S4. (True)

PRACTICE SOLUTIONS

S1. (b) Restriction enzymes cut DNA creating sticky ends of restriction fragments. While DNA ligase joins these sticky ends to form recombinant DNA.

S2. (b) cutting and extraction of DNA is done to separate the DNA from the gel in which it is collected. Water or a low salt buffer is added to break the cation bridge and dislodge the DNA from the gel and elute it.

S3. (c) The cloning vector requires the presence of a selectable marker, which helps in identifying and eliminating non-transformants and selectively permitting the growth of the transformants. Normally, the genes encoding resistance to antibiotics such as ampicillin, chloramphenicol, tetracycline or kanamycin, etc. are considered useful selectable markers for E. coli. The normal E.coli cells do not carry resistance against any of these antibiotics.

S4. (d)

S5. (d) During this stage the cocktail containing the template DNA and all the other core ingredients is heated to 94-95^{0}C.

S6. (c) the most commonly used matrix is agarose which is a natural polymer extracted from seaweeds.

S7. (c) the presence of a chromogenic substrate gives blue coloured colonies if the plasmid in the bacteria does not have an insert. Presence of insert results into insertional inactivation of the -galactosidase and the colonies do not produce any colour, these are identified as recombinant colonies.

S8. (d) he presence of a chromogenic substrate gives blue coloured colonies if the plasmid in the bacteria does not have an insert. Presence of insert results into insertional inactivation of the -galactosidase and the colonies do not produce any colour, these are identified as recombinant colonies.

S9. (d) Ori site or the site of origin controls replication in circular plasmid DNA and hence, the copy number of linked DNA in the vector.

S10. (b) the protein is called a recombinant protein.

S11. (a) such group of letters are called palindrome.

S12. (a) exonucleases are the enzymes that are necessary for proofreading and removing the nucleotides from the DNA ends and the endonucleases are the enzymes that cut DNA at specific positions.

S13. (c) removal of RNA can be done by ribonucleases and proteins can be done by protease.

S14. (a) The Roman numerals are used to identify specific enzymes from bacteria that contain multiple restriction enzymes. Typically, the Roman numeral indicates the order in which restriction enzymes were discovered in a particular strain.

S15. (a) Key tools for recombinant DNA technology are restriction endonuclease enzyme and DNA ligases for recombinant DNA production, vectors that help in carrying and integrating the desired gene, host organism is that one in which recombinant DNA is introduced, polymerase enzymes

S16. (b) In the cloning vector pBR322, ampicillin and tetracycline resistance genes are the selectable markers. The role they play is that they help in the selection of transformed cells from non transformed cells. They also help distinguish recombinant cells from non-recombinant cells.

S17. (d) they were restriction endonucleases.

S18. (c) the denaturation step will be affected.

S19. (b) the DNA stained with ethidium bromide when viewed under UV light appear bright orange.

S20. (a) Polymerase chain reaction or PCR consists of the following three steps:
Denaturation- The two DNA strands of template DNA separate from each other when heated to 92°C, next is annealing- The primers anneal to the 3' end of single strands of DNA and the last is extension- The primers are extended by DNA polymerase by the addition of nucleotides to form complete strands of DNA. Hence the sequence of steps is denaturation, annealing, extension.

S21. (a) a patent is a, intellectual property right that provides the owner the legal right to ensure that no one else benefits from making, using or selling an invention.

S22. (d) Vectors carry an antibiotic-resistant gene which helps to select the recombinant cells from non-recombinant ones as only recombinant cells would exhibit the antibiotic resistance and would be able to survive on antibiotic rich medium. Such genes that the host cell requires for growth under certain conditions and differentiate the recombinant cells from non-recombinant ones are called as selectable markers.

S23. (c) in electrophoresis, the negatively charged DNA moves from negative terminal to the positive terminal.

S24. (a) Sticky ends contain free or hanging or unpaired nitrogen bases which can pair to complementary bases present on other DNA segment required to create a recombinant DNA. A ligase is required in absence of sticky ends to join together two segments of DNA.

S25. (a) Only those cells which have been transformed successfully can be traced by marker. So, this helps in eliminating non-transformants and permitting transformants.

S26. (d) In recombinant DNA technology, selectable markers are the specific genes that are used to identify the transformants from the non-transformants after the process of recombination. These genes are used to detect whether the incorporation of a nucleic acid sequence has been successful into an organism's DNA.

S27. (a) A restriction enzyme or restriction endonuclease is an enzyme that cleaves DNA into fragments at or near specific recognition sites within the molecule known as restriction sites. Restrictions enzymes are one class of the broader endonuclease group of enzymes.

S28. (c) Plasmids are autonomously replicating circular extra-chromosomal DNA. They are the standard cloning vectors and the ones most commonly used. Most general plasmids may be used to clone DNA insert of up to 15 kb in size.

S29. (d) The biolistic method is a method of transformation of plants. It is used to transfer foreign DNA into a plant cell. For the purpose, cells are bombarded with high-velocity micro-particles of gold or tungsten coated with DNA.

S30. (b)

ASSERTION AND REASON

S1. (a)

S2. (b)

S3. (d) Exonucleases remove nucleotides from the ends of the DNA.

S4. (b)