Very Short Answer Questions (PYQ)

[1 Mark]

Q.1. Write the two components of the first artificial recombinant DNA molecule constructed by Cohen and Boyer.

Ans. The two components were—antibiotic resistance gene and plasmid vector of *Salmonella typhimurium*.

Q.2. What is the host called that produces a foreign gene product? What is this product called?

Ans. The host that produces a foreign gene product is called competent host. The product is called recombinant protein.

Q.3. Mention the uses of cloning vector in biotechnology.

Ans. Cloning vectors are used for transferring fragments of foreign DNA into a suitable host. They are also used to select recombinants from non-recombinants.

Q.4. How does an alien DNA gain entry into a plant cell by 'biolistics' method?

Ans. In biolistics method, cells are bombarded with high velocity micro-particles of gold or tungsten coated with DNA.

Q.5. How is the action of exonuclease different from that of endonuclease?

Ans. Exonucleases cleave the DNA molecules at their ends whereas endonucleases cleave DNA molecules internally.

Q.6. Why is it essential to have a 'selectable marker' in a cloning vector?

Ans. Selectable markers are essential to identify and eliminate non-transformants, by selectively permitting the growth of the transformant.

Q.7. Biotechnologists refer to *Agrobacterium tumifaciens* as a natural genetic engineer of plants. Give reasons to support the statement.

Ans. This is because A. *tumifaciens* can transfer genes naturally by delivering a piece of T-DNA to plant cells. It has a tumour inducing plasmid.

Q.8. Why is the enzyme cellulase needed for isolating genetic material from plant cells and not from the animal cells?

Ans. The enzyme cellulase breaks down cellulose which is present in cell walls of plants but absent in animal cells.

Q.9. Suggest a technique to a researcher who needs to separate fragments of DNA.

Ans. Gel electrophoresis is used to separate DNA fragments.

Q.10. Mention the type of host cells suitable for the gene guns to introduce an alien DNA.

Ans. Plant cells.

Q.11. Why is it not possible for an alien DNA to become part of a chromosome anywhere along its length and replicate normally?

Ans. Alien DNA must be linked to ori or origin of replication site to start replication.

Q.12. Name the host cells in which micro-injection technique is used to introduce an alien DNA.

Ans. Animal cells.

Q.13. Write the name of the enzymes that are used for isolation of DNA from bacterial and fungal cells respectively for Recombinant DNA technology.

Ans. Bacterial cell is treated with enzyme lysozyme.
Fungal cell is treated with chitinase.

Q.14. How is repetitive/satellite DNA separated from bulk genomic DNA for various genetic experiments?

Ans. By density gradient centrifugation.

Q.15. How can bacterial DNA be released from the bacterial cell for biotechnology experiments?

Ans. The bacterial cell wall is digested by the enzyme lysozyme to release DNA from the cell.

Very Short Answer Questions (OIQ)

[1 Mark]

Q.1. What is biotechnology?

Ans. Biotechnology is the technological employment of living organisms (especially micro-organisms) to produce the materials for human uses.

Q.2. Expand EFB?

Ans. European Federation of Biotechnology

Q.3. What is the function of restriction enzyme?

Ans. To cut DNA at specific site.

Q.4. Name the first plasmid used as vector.

Ans. pBR322.

Q.5. What are palindromes?

Ans. Palindromes are group of letters (sequences) that read same both in forward and backward direction.

Q.6. What is cloning vector?

Ans. They are carriers or vehicles of desired DNA fragments which can replicate independently to increase copies of desired genes in the host cell, e.g., plasmids, bacteriophages.

Q.7. Expand the terms: *r*DNA, BACs, YACs.

Ans. *r*DNA—Recombinant DNA
BACs—Bacterial Artificial Chromosomes
YACs—Yeast Artificial Chromosomes

Q.8. What is recombinant DNA?

Ans. Recombinant DNA is the DNA formed by combining DNAs from two different organisms.

Q.9. What is a plasmid?

Ans. A plasmid is a circular extra-chromosomal DNA molecule present in a bacterial cell, which replicates autonomously, of the bacterial chromosomal DNA.

Q.10. Name the substance used as a medium/matrix in gel electrophoresis.

Ans. Agarose.

Q.11. What is gene gun?

Ans. The instrument for bombarding micro-projectile particles (gold/tungsten particles) coated with foreign DNA, with great velocity, into a target cell is called gene gun.

Q.12. Name the compound used for staining the isolated DNA in the gel electrophoresis.

Ans. Ethidium bromide.

Q.13. Name the technique used for separating DNA fragments in the laboratory.

Ans. Gel electrophoresis.

Q.14. Who developed the technique of electrophoresis? Write the principle on which it is based.

Ans. Electrophoresis was developed by Tiselius in 1937 and is based on the principle that charged particles move under the influence of electric current to oppositely charged electrodes.

Q.15. What is a bioreactor?

Ans. It is generally a 100–1000 litre cylindrical metal container with a curved base to facilitate mixing of contents. In bioreactors, raw materials are biologically converted into products.

Q.16. Which enzyme is used to digest walls of bacteria and fungi in genetic engineering?

Ans. Lysozyme digests bacterial wall and chitinase digests fungal cell wall.

Q.17. Why does DNA move towards the anode in gel electrophoresis?

Ans. The DNA fragments are negatively charged so they move towards the positively charged anode.

Q.18. Why is a thermostable DNA polymerase needed in amplification (genetic engineering)?

Ans. Because thermostable DNA polymerase remains active even at high temperature required for extension step of PCR.

Q.19. A technique used in making copies of a specific segment of DNA involves

i. ligase chain reaction
ii. transcription
iii. polymerase chain reaction
iv. translation.

Ans. (*iii*) polymerase chain reaction.

Q.20. "The prophase I of meiosis plays a vital role in rDNA formation" Justify the statement.

Ans. This is because crossing over occurs in this phase which helps to produce recombinants.

Q.21. Eukaryotes do not have restriction endonuclease, then how they manage with normal endonuclease enzyme?

Ans. In eukaryotes, normal endonuclease attach with special proteins, like initiator/terminator, to perform their functions.

Q.22. Identify the reason for selection of DNA polymerase from *Thermus aquaticus* for Polymerase Chain Reaction.

Ans. DNA polymerase from *Thermus aquaticus* remains active during the high temperature induced denaturation of double stranded DNA.

Q.23. Why EtBr is used in gel electrophoresis inspite of it being highly carcinogenic?

Ans. Ethidium bromide (EtBr) exchanges its visible range of wavelength with the invisible wavelength of DNA to make it visible under UV light.

Q.24. How is the action of restriction endonucleases different from that of normal endonucleases?

Ans. Normal Endonuclease makes cuts at random positions within a DNA sequence. Restriction endonuclease makes cuts only at specific positions within a DNA sequence.

Q.25. Give any two microbes that are useful in biotechnology.

Ans. *E. coli* and *Saccharomyces cerevisiae.*

Q.26. Which main technique and instrument is used to isolate DNA from any plant cell?

Ans. Centrifugation and centrifuge

Q.27. Expand the following:

i. *c*DNA
ii. Bt

Ans.

i. *c*DNA—Complementary DNA
ii. Bt—*Bacillus thuringiensis*

Short Answer Questions-I (PYQ)

[2 Marks]

Q.1. What is *Eco*RI? How does *Eco*RI differ from an exonuclease?

Ans. *Eco*RI is restriction endonuclease enzyme. Exonuclease removes nucleotides form the ends of DNA while *Eco*RI makes cut at specific position within the DNA.

Q.2. Explain with the help of an example the relationship between restriction endonuclease and a palindromic nucleotide sequence.

Ans. Restriction endonuclease recognises a specific palindromic nucleotide sequence in the DNA. Restriction endonuclease cuts the strand of DNA a little away from the centre of palindromic nucleotide sequence but between the same two bases on the opposite strands, leaving single stranded portions at the end or sticky ends.

Q.3. Explain palindromic nucleotide sequence with the help of a suitable example.

Ans. The palindrome in DNA is a sequence of base pairs that reads same on the two strands when orientation of reading is kept the same. For example, the following sequences reads the same on the two strands in 5′ → 3′ direction. This is also true if it is read in the 3′ → 5′ direction.

5′ — — GAATTC — — 3′
3′ — — CTTAAG — — 5′

Q.4.

a. **What are "molecular scissors"? Give one example.**
b. **Explain their role in recombinant DNA technology.**

OR

Why are molecular scissors so called? Write their use in biotechnology.

Ans.

a. The restriction endonucleases are called molecular scissors, as they cut the DNA segments at particular locations, *e.g.*, *Eco*RI.
b. The restriction enzymes cut the DNA strands a little away from the centre of the palindromic sites, but between the same two bases on the opposite strands. This

leaves single stranded portions with overhanging stretches called sticky ends on each strand as they form hydrogen bonds with their complementary cut counterparts. This stickiness at the ends facilitates the action of the enzyme DNA ligase.

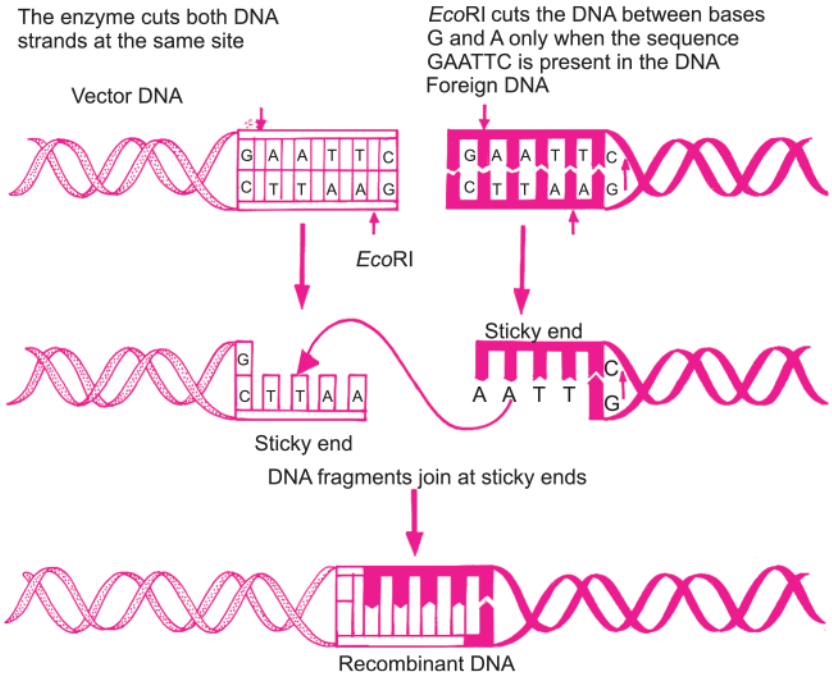
Q.5. Explain the role of the enzyme *EcoRI* in recombinant DNA technology.

OR

Explain the role of restriction endonucleases in recombinant DNA technology. Name the endonuclease that was first discovered.

Ans. *Eco*RI inspects length of DNA and recognises specific palindromic nucleotide sequences. It then binds with DNA and cuts each of the two strands of double helix at specific points.

Mechanism of Action of Endonucleases



Steps in formation of recombinant DNA by action of restriction endonuclease enzyme *EcoRI*

- Every endonuclease inspects the entire DNA sequence for the palindromic recognition sequence.
- On finding the palindrome, the endonuclease binds to the DNA.
- It cuts the opposite strands of DNA in the sugar–phosphate backbone; a little away from the centre of the palindrome sites but between the same bases on both strands.
- This results in the formation of single stranded overhanging stretches at the end of each strand called sticky ends.

- The sticky ends facilitate the action of the enzyme DNA ligase by readily forming hydrogen bonds with complementary strands.
- In genetic engineering, DNA from different sources are cut with the same restriction enzymes so that both DNA fragments have same kind of sticky ends.
- These sticky ends are complementary to each other and thus can be joined by DNA ligase (end-to-end).

The first endonuclease discovered was *Hin*dII.

Q.6. Write the convention used for naming restriction enzymes.

OR

Explain with the help of a suitable example the naming of a restriction endonuclease.

Ans. The convention for naming restriction enzymes is that the first letter to the name comes from the Genus and the second two letters come from species and third letter indicates the strain of the prokaryotic cell from which they are isolated *e.g., Eco*RI comes from *Escherichia coli* RI, here R stands for the strain and I follows the order in which the enzyme was isolated.

Q.7. Name the natural source of agarose. Mention one role of agarose in biotechnology.

Ans. The natural source of agarose is sea weed. Agarose is a natural polymer. It is used to develop the matrix for gel electrophoresis. It helps in the separation of DNA fragments based on their size.

Q.8. Write any four ways used to introduce a desired DNA segment into a bacterial cell in recombinant technology experiments.

Ans.

i. The desired DNA segment is inserted into a cloning vector and the bacterial cell can be made to take it up after making them competent by treating them with specific concentration of divalent cations such as calcium.
ii. Microinjection
iii. Biolistics
iv. Disarmed pathogen vector

Q.9. What are 'cloning sites' in a cloning vector? Explain their role. Name any two such sites in pBR322.

Ans. Cloning sites are the recognition sites on plasmid. The restriction enzymes recognise these sites for cutting and ligation of alien DNA at this place. For example, *Eco*RI, *Bam*HI.

Q.10.

a. **Mention the difference in the mode of action of exonuclease and endonuclease.**
b. **How does restriction endonuclease function?**

Ans.

a. Exonuclease removes nucleotides from the ends of DNA whereas endonuclease cuts at specific positions within DNA at specific positions.
b. Restriction endonuclease recognises and cuts specific palindromic nucleotide sequences in the DNA.

Q.11. How does a restriction nuclease function? Explain.

Ans. Restriction nuclease cuts DNA at specific sites. Nucleases are of two types exonuclease and endonuclease.
Exonuclease cuts DNA at the ends, whereas endonuclease cuts at specific sites within DNA.

Q.12. How are 'sticky ends' formed on a DNA strand? Why are they so called?

Ans. Restriction enzymes cut the strands of the DNA, a little away from the centre of the palindromic sites, but between the same two bases on opposite strands. This leaves called sticky single stranded position at the ends. These overhanging stretches are aids. These are named so because they form hydrogen bonds with their complementary cut counterparts.

Q.13. How can DNA segments, separated by gel electrophoresis, be visualised and isolated?

Ans. The separated DNA molecules are visualised only after staining DNA with ethidium bromide followed by exposure to UV radiation. They appear as bright orange coloured bands. The separated bands of DNA (on the gel) are cut from the agarose gel and extracted from the gel piece. This process is called elution.

Q.14. Why does the 'insertional inactivation' method to detect recombinant DNA is preferred to 'antibiotic resistance' procedure?

Ans. In insertional inactivation method, the presence of a chromogenic substrate gives blue coloured colonies in absence of an insert. Presence of an insert in the enzyme site do not produce colour. This is because insertional inactivation of the β-galactosidase has taken place due to the insert. Antibiotic resistance method requires duplicate plating. It is a cumbersome procedure to perform.

Q.15. Why and how bacteria can be made 'competent'?

Ans. Bacteria are made competent to accept the DNA or plasmid molecules. This is done by treating them with specific concentration of a divalent cation such as calcium to increase pore size in cell wall. The cells are then incubated with recombinant DNA on ice, followed by placing them briefly at 42°C and then putting it back on ice.

Q.16. Name the source of the DNA polymerase used in PCR technique. Mention why it is used.

Ans. The source is the bacterium *Thermus aquaticus*. It is used because it is thermostable and do not denature at high temperatures.

Q.17. Name the source organism that possesses *Taq* polymerase. What is so special about the function of this enzyme?

OR

Name the organism from where the thermostable DNA polymerase is isolated. State its role in genetic engineering.

Ans. Source organism: *Thermus aquaticus*
The enzyme can tolerate high temperature and is thus thermostable. It does not get denatured during PCR at high temperature.

Q.18. How are recombinant vectors created? Why is only one type of restriction endonuclease required for creating one recombinant vector?

Ans. The construction of recombinant DNA is done by linking a gene encoding antibiotic resistance with a native plasmid. These plasmid DNA act as vectors to transfer the piece of DNA attached to it.
Only one type of restriction endonuclease is required for creating recombinant vector because when cut by the same enzyme, the resultant DNA fragments have the same sticky ends, which can be joined together using DNA ligases.

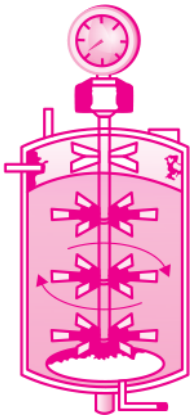
Q.19. How is DNA isolated in purified form from a bacterial cell?

Ans. DNA, a genetic material is isolated in purified form by treating the bacterial cells with the enzymes such as lysozyme to remove the cell wall. The RNA thus released can be removed by treating them with ribonuclease and enzyme proteases is added to remove proteins. Finally, chilled ethanol is added to precipitate the purified DNA.

Q.20. What are recombinant proteins? How do bioreactors help in their production?

Ans. The protein produced by genetically altered gene in a host is called recombinant protein. Bioreactors are vessels in which raw materials are biologically converted into specific products by microbes. It provides optimum growth conditions such as temperature, pH, substrate, vitamins, oxygen and salts.

Q.21. Name the type of bioreactor shown. Write the purpose for which it is used.



Ans. The given bioreactor is the simple stirred tank bioreactor.
Its purpose is large scale production of recombinant protein or enzymes, using microbial plants/ animals/human cells.

Q.22.

a. **Explain how to find whether an E. coli bacterium has transformed or not when a recombinant DNA bearing ampicillin resistant gene is transferred into it.**
b. **What does the ampicillin resistant gene act as in the above case?**

Ans.

a. *E. coli* bearing transferred recombinant DNA are first grown on ampicillin containing medium and then transferred on to a medium containing tetracycline. The transformants will grow only in ampicillin containing medium and not in tetracycline containing medium. The non-transformants, on the other hand, will grow in both the mediums.
b. Ampicillin resistant gene acts as a selectable marker and helps in selecting the transformants.

Q.23. Write the role of '*ori*' and 'restriction' site in a cloning vector pBR322.

Ans. *ori* is the site where replication starts. This site is responsible for controlling the copy number a vector.

Restriction site is the site of ligation of alien/foreign DNA in the vector, in one of the two **antibiotic resistance site or coding sequence of α-galactosidase.**

Q.24.

a. **A recombinant vector with a gene of interest inserted within the gene of α-galactosidase enzyme, is introduced into a bacterium. Explain the method**

that would help in selection of recombinant colonies from non-recombinant ones.

b. Why is this method of selection referred to as "insertional inactivation"?

Ans.

a. Bacteria is grown in a medium with chromogenic substrate, blue coloured colonies show no recombinations and colonies with no blue colour show presence of recombinants.
b. Gene for the enzyme is inactivated by insertion of foreign DNA.

Q.25. How is insertional inactivation of an enzyme used as a selectable marker to differentiate recombinants from non-recombinants?

Ans. When a recombinant DNA is inserted within the coding sequence of an enzyme β-galactosidase, it results into inactivation of the enzyme. The bacterial colonies having inserted plasmid, show no colouration while those without inserted plasmid show blue colour.

Q.26. Rearrange the following in the correct sequence to accomplish an important biotechnological reaction:

a. *In vitro* synthesis of copies of DNA of interest
b. Chemically synthesised oligonucleotides
c. Enzyme DNA-polymerase
d. Complementary region of DNA
e. Genomic DNA template
f. Nucleotides provided
g. Primers
h. Thermostable DNA-polymerase (from Thermus aquaticus)
i. Denaturation of *ds*DNA

Ans. Correct sequence is

i → e → b/g → g/b → c/b → h/c → f → d → a

Q.27. Name the source organism from which *Ti* plasmid is isolated. Explain the use of this plasmid in biotechnology.

Ans. *Ti* plasmid is isolated from *Agrobacterium tumifaciens*. *Ti* plasmid of *Agrobacterium tumifaciens* has been modified into a cloning vector, which is not pathogenic to plants but still is able to use the mechanisms to deliver genes of interest into plants.

Q.28. Explain the work carried out by Cohen and Boyer that contributed immensely in biotechnology.

Ans. Stanley Cohen and Herbert Boyer in 1972 constructed the first recombinant DNA. They isolated the antibiotic resistance gene by cutting out a piece of DNA from the plasmid of a bacterium which was responsible for conferring antibiotic resistance. The cut piece of DNA was then linked with the plasmid DNA of *Salmonella typhimurium* and transferred to E. coli for transformation.

Q.29. State how has Agrobacterium tumefaciens been made a useful cloning vector to transfer DNA to plant cells.

Ans. *Agrobacterium tumifaciens* is known to be a natural vector and consists of a pathogenic plasmid. It is capable of passing its DNA to plants and induce tumour by integrating its DNA with host genome. The tumour causing gene in the plasmid of this bacteria is replaced by gene of interest and is now used as a cloning vector to transfer the DNA into plant cells.

Q.30. How can the following be made possible for biotechnology experiments?

a. **Isolation of DNA from bacterial cell.**
b. **Reintroduction of the recombinant DNA into a bacterial cell.**

Ans.

a. By treating cell with lysozyme
b. Microinjection/gene gun

Short Answer Questions-I (OIQ)

[2 Mark]

Q.1. Define genetic engineering. Name one natural genetic engineer of plants.

Ans. Genetic engineering is the manipulation of genetic materials which can be introduced into host organisms and thus change the phenotype of the host organism.

The natural genetic engineer of plant is *Agrobacterium tumifaciens*.

Q.2. Explain any two methods of vector less gene transfer.

Ans. The two methods of vectorless gene transfer are:

i. **Micro-injection:** The technique of introducing foreign gene in a target cell by injecting the DNA, directly into the nucleus, by micro-needle is called micro-injection.
ii. **Electroporation:** It is the process in which transient holes are produced in the plasma membrane of the target cell, to incorporate foreign DNA.

Q.3. What is meant by gene cloning?

Ans. Gene cloning refers to a process in which a gene of interest is ligated to a vector. The recombinant DNA thus produced is introduced in a host cell by transformation. Each cell gets one DNA molecule and when the transformed cell grows to a bacterial colony, each cell in the colony has a copy of the gene.

Q.4. What is the role of lysing enzyme in biotechnology?

Ans. Lysing enzymes are used to open up the cell to obtain DNA along with other macromolecules for genetic experiments. Bacterial cells are treated with lysozyme, plant cells are treated with cellulase, and fungal cells are treated with chitinase for lysing.

Q.5. What do you mean by a clone?

Ans. The cell or organism derived from the same parents by asexual mean which are genetically identical to each other and to the parent are called clones.

Q.6. What is elution?

Ans. The separated band of DNA (after gel electrophoresis) are cut out from agarose gel and DNA is extracted from the gel piece. This step is called elution.

Q.7. Differentiate between *r*DNA and *c*DNA.

Ans.

***r*DNA**	***c*DNA**
It is the DNA which is formed by joining together the DNA from two different organisms.	It is the DNA which is obtained from RNA template catalysed by reverse transcriptase enzyme.

Q.8. Why are cloning vectors necessary in cloning? Name any two such vectors that are used in experiment with *E. coli*.

Ans. DNA being hydrophilic in nature cannot pass through the cell membranes into the host. Therefore, cloning vectors are required to transfer the DNA into the host by attaching the desired DNA to it.
The two cloning vectors that are used are plasmids and bacteriophages.

Q.9. Why is *Agrobacterium tumifaciens* a good cloning vector? Explain.

Ans. *Agrobacterium tumifaciens* is a soil bacterium which causes disease in many dicot plants. It is able to deliver a piece of DNA known as T-DNA, to transform the normal cells into tumour cells and direct these tumour cells to produce the chemicals required by the pathogen. The tumour inducing (Ti) plasmid of *Agrobacterium tumifaciens* has

now been modified into a cloning vector which is no more pathogenic to the plants but still deliver genes of interest into a variety of plants.

Q.10. Why is *Agrobacterium*-mediated genetic transformation described as natural genetic engineering in plants?

Ans. *Agrobacterium tumifaciens* is a pathogen of several dicot plants which exhibit natural genetic engineering in plant.

Reasons:

i. It is able to deliver a piece of DNA called 'T-DNA' to transform normal plant cell into a tumour cell.
ii. The DNA transforms the normal cells into tumour cells which direct them to produce the chemical essential for the pathogen.

As this process occurs in nature it is called natural genetic engineering.

Q.11. When a foreign DNA is introduced into an organism, how is it maintained in the host and how is it transferred to the progeny of the organism?

Ans. Foreign gene is usually ligated to a plasmid vector and introduced in the host. As plasmid replicates and makes multiple copies of itself, foreign gene also gets replicated and its copies are also made. When the host organism divides, its progeny also receives the plasmid DNA containing the foreign gene.

Q.12. What modification is done in the *Ti* plasmid of *Agrobacterium tumefaciens* to convert it into a cloning vector?

Ans. T-DNA is the only essential part required to make *Ti* plasmid a cloning vector. The plasmid is disarmed by deleting the tumour inducing genes in the plasmid so that it becomes an effective cloning vector and remove it harmful effect.

Q.13. What does 'competent' refer to in competent cells used in transformation?

Ans. Competent means bacterial cells which by various methods like treatment with $CaCl_2$ are made capable of taking up foreign DNA.

Q.14. Describe the role of $CaCl_2$ in preparation of competent cells.

Ans. $CaCl_2$ is known to increase the efficiency of DNA uptake to produce transformed bacterial cells. The divalent Ca^{2+} ions supposedly create transient pores in the bacterial cell wall, by which the entry of foreign DNA is facilitated into the bacterial cells.

Q.15. What is the significance of adding proteases at the time of isolation of genetic material (DNA)?

Ans. Role of proteases is to degrade the proteins present inside a cell (from which DNA is being isolated). If the proteins are not removed from DNA preparation then they could interfere with any downstream treatment of DNA.

Q.16. While doing a PCR, 'denaturation' step is missed. What will be its effect on the process?

Ans. If denaturation of double-stranded DNA does not take place, then primers will not be able to anneal to the template, no extension will take place, hence no amplification will occur.

Q.17. Illustrate briefly downstream processing.

Ans. Downstream processing

- All the processes to which a product is subjected to before being marketed as a finished product are called downstream processing.
- It includes:
    a. Separation of the product from the reactor.
    b. Purification of the product.
    c. Formulation of the product with suitable preservatives.
    d. Quality control testing and clinical trials in case of drugs.

Q.18. What would happen when you grow a recombinant in a bioreactor but forget to add antibiotic to the medium in which the recombinant is growing?

Ans. In the absence of antibiotic, there will be no pressure on recombinants to retain the plasmid (containing the gene of your interest). Since, maintaining a high copy number of plasmids is a metabolic burden to the microbial cells, it will thus tend to lose the plasmid.

Q.19. A wine maker and a molecular biologist who has developed a recombinant vaccine, both claim themselves to be biotechnologist. Who in your opinion is right?

Ans. Both are right because biotechnology is a very wide area which deals with techniques of using a 'natural' organism (or its parts) as well as genetically modified organism to produce products and processes useful for mankind. A wine maker employs a strain of yeast to produce wine by fermentation (a natural phenomenon), while the molecular biologist has cloned gene for the antigen (that is used as vaccine) in an organism which allows the production of the antigen in large amount.

Q.20. For producing a recombinant protein (for therapeutic purpose) in large scale, which vector would you choose—a low copy number or high-copy number?

Ans. High-copy number, because higher the copy number of vector plasmid, higher the copy number of gene and consequently, protein coded by the gene is produced in high amount.

Q.21. Would you like to choose an exonuclease enzyme while producing a recombinant DNA molecule?

Ans. No, as exonuclease acts on the free ends of linear DNA molecule. Therefore, instead of producing DNA fragments with sticky ends, it will shorten or completely degrade the DNA fragment containing the gene of interest, and the circular plasmid (vector) will not get cut as it lacks free ends.

Q.22. You have created a recombinant DNA molecule by ligating a gene to a plasmid vector. By mistake, your friend adds exonuclease enzyme to the tube containing the recombinant DNA. How will your experiment get affected as you plan to go for transformation now?

Ans. The experiment will not likely be affected as recombinant DNA molecule is circular and closed, with no free ends. Hence, it will not be a substrate for exonuclease enzyme which removes nucleotides from the free ends of DNA.

Q.23. DNA being hydrophilic cannot pass through the cell membrane of a host cell. Explain how does recombinant DNA get introduced into the host cell to transform the latter.

Ans. The cell is made competent by the following methods:

a. **Chemical method**

- The cell is treated with specific concentration of a divalent cation such as calcium to increase pore size in cell wall.
- The cells are incubated with recombinant DNA on ice, followed by placing them briefly at 42°C and then putting it back on ice. This is called heat shock treatment.
- The bacteria now take up the recombinant DNA.

b. **Physical methods**

The physical methods include

- **Micro-injection method:** Recombinant DNA is directly injected into the nucleus of an animal cell.
- **Biolistics or gene gun method:** Cells are bombarded with high velocity micro-particles of gold or tungsten coated with DNA in plants.

Q.24. PCR is a useful tool for early diagnosis of an infectious disease. Comment.

Ans. PCR is a very sensitive technique which enables the specific amplification of desired DNA from a limited amount of DNA template. Hence, it can detect the presence of an infectious organism in the infected patient at an early stage of infection (even before the infectious organism has multiplied to large number).

Q.25. Restriction enzymes present in the cloning site of a vector should not have more than one recognition site. Comment.

Ans. If the restriction enzymes have more than one recognition site in a vector, then the vector itself will get fragmented on treatment with the restriction enzyme.

Q.26. You have chosen a plasmid as vector for cloning your gene. However this vector plasmid lacks a selectable marker. How would it affect your experiment?

Ans. In a gene cloning experiment, first a recombinant DNA molecule is constructed, where the gene of interest is ligated to the vector and introduced inside the host cell (transformation). Since, not all the cells get transformed with the recombinant/plasmid DNA, in the absence of selectable marker, it will be difficult to distinguish between transformants and non-transformants, because role of selectable marker is in the selection of transformants.

Q.27. Write the use of the following in biotechnology.

Q. Chilled ethanol

Ans. It is added to precipitate the purified DNA to isolate it.

Q. Microinjection

Ans. It is used to inject the foreign gene into a host cell, directly.

Q. Bioreactor

Ans. It is the set up to culture large volumes of transgenic bacteria to get large quantities of the product protein.

Q. Plasmid

Ans. It is the vector to transform a foreign gene.

Q.28. Is there any difference between recombinant DNA and recombinant protein? Support your answer.

Ans. *r*DNA is the plasmid vector containing the foreign DNA whereas recombinant protein is the product of transgenic gene in the host body or cell.

Q.29. Where and why do we use *Taq* polymerase enzyme when it works exactly as DNA polymerase?

Ans. In PCR, because it is a thermostable DNA polymerase enzyme, gets isolated from bacteria Thermus aquaticus from hot water springs, and it does not get denatured at high temperature which is required during PCR and works as normal DNA polymerase enzyme (whereas the normal DNA polymerase gets denatured at high temperature).

Q.30. A vector is engineered with three features which facilitate its cloning within the host cell. List the three features and explain each one of them.

Ans.

i. **Origin of replication/*ori* site—**From here the replication starts (and any piece of DNA when linked, can be made to replicate within the host cell).
ii. **At least two Selectable markers—**Helpful in identifying and eliminating non-transformants.
iii. **Unique Restriction sites for more than one restriction enzymes—**The foreign DNA links to this region of the plasmid.

Short Answer Questions-II (PYQ)

[3 Marks]

Q.1. List the steps involved in *r*DNA technology.

Ans. Steps in *r*DNA technology:

i. Isolation of DNA.
ii. Fragmentation of DNA by restriction endonucleases.
iii. Isolation of the desired DNA fragments.
iv. Amplification of the gene of interest.
v. Ligation of the DNA fragment into a vector using DNA ligase.
vi. Transfer of recombinant DNA into the host organism.
vii. Culturing the host cell on a suitable medium on a large scale.
viii. Extraction of the desired product.
ix. Downstream processing of the products as finished products are ready for marketing.

Q.2. List the key tools used in recombinant DNA technology.

Ans. The key tools used in recombinant DNA technology are:

i. Restriction enzymes
ii. Polymerase enzyme
iii. Ligase enzyme
iv. Vectors
v. Host organism/cell.

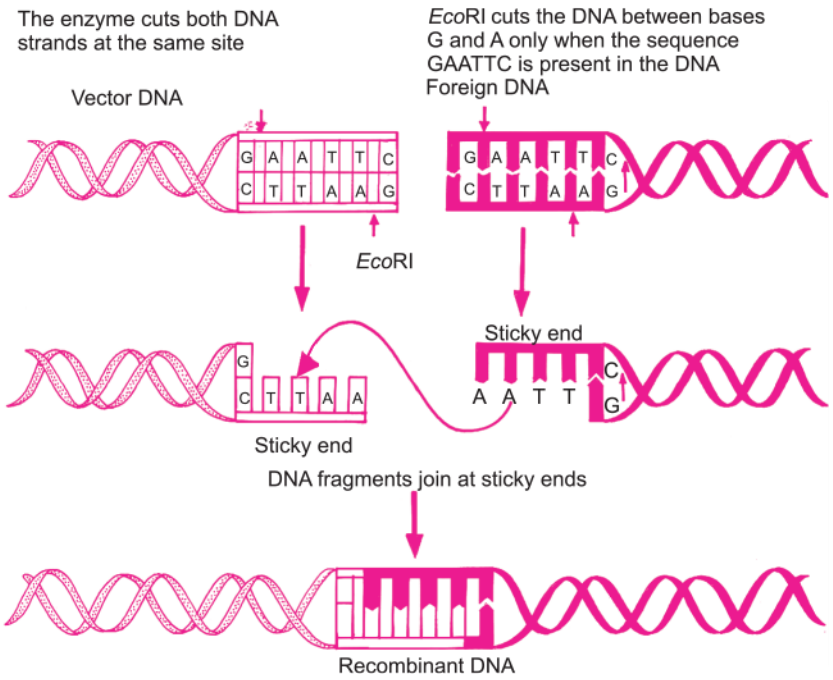
Q.3. Explain the action of the restriction endonuclease *Eco*RI.

Ans.

i. The recognition sequence shows palindrome character in which the sequence of base pairs read the same on both the DNA strands, *i.e.*, same in 5′ → 3′ or 3′ → 5′ directions, *e.g.*,
5′ — G A A T T C — 3′
3′ — C T T A A G — 5′
ii. The restriction endonuclease acts on specified length of a DNA and binds to the DNA at the recognition sequence.
iii. It cuts the opposite double helix of DNA in the sugar-phosphate backbones, at little away from the centre of the palindrome sites.
iv. There are overhanging stretches called sticky ends on each strand, which form hydrogen bonds with their complementary cut counterparts. This stickiness of the ends facilitates the action of the enzyme DNA ligase.

Q.4. Prepare a flow chart in formation of recombinant DNA by the action of restriction endonuclease enzyme *Eco*RI.

Ans.



Steps in formation of recombinant DNA by action of restriction endonuclease enzyme *EcoRI*

Q.5.

a. **What is *Eco*RI? What does 'R' represent in this?**
b. **Explain its action.**

Ans.

a. *Eco*RI is a restriction endonuclease, obtained from an *E. coli* bacterium. R represents the name of the strain.
b. It cuts the DNA between bases G and A on both the strands only when the sequence GAATTC is present in DNA.

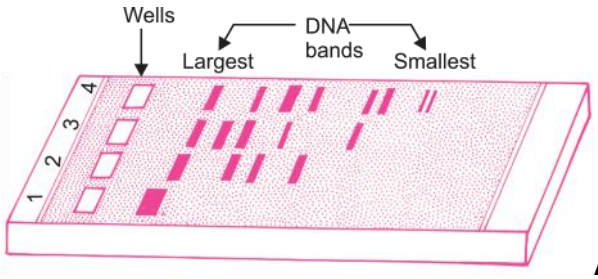
Q.6. Name and explain the technique used for separating DNA fragments and making them available for biotechnology experiments.

OR

How are the DNA fragments separated and isolated for DNA fingerprinting? Explain.

Ans. Separation and Isolation of DNA Fragments (Gel Electrophoresis)

- Gel electrophoresis is a technique for separating DNA fragments based on their size.
- Firstly, the sample DNA is cut into fragments by restriction endonucleases.
- The DNA fragments being negatively charged can be separated by forcing them to move towards the anode under an electric field through a medium/matrix.
- Commonly used matrix is agarose, which is a natural linear polymer of D-galactose and 3, 6-anhydro-L-galactose which is extracted from sea weeds.
- The DNA fragments separate-out (resolve) according to their size because of the sieving property of agarose gel. Hence, smaller the fragment size, the farther it will move.



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

- The separated DNA fragments are visualised after staining the DNA with ethidium bromide followed by exposure to UV radiation.
- The DNA fragments are seen as orange coloured bands.
- The separated bands of DNA are cut out and extracted from the gel piece. This step is called elution.
- The purified DNA fragments are used to form recombinant DNA which can be joined with cloning vectors.

Q.7. Explain the process by which a bacterial cell can be made 'competent'. Why is it essential to make bacterial cells 'competent' in recombinant DNA technology?

OR

How can be a host made competent? Explain the different methods.

Ans. The cell is made competent by the following methods:

a. **Chemical method**
- The cell is treated with specific concentration of a divalent cation such as calcium to increase pore size in cell wall.
- The cells are incubated with recombinant DNA on ice, followed by placing them briefly at 42°C and then putting it back on ice. This is called heat shock treatment.
- The bacteria now take up the recombinant DNA.

b. **Physical methods**
The physical methods include

- **Micro-injection method:** Recombinant DNA is directly injected into the nucleus of an animal cell.
- **Biolistics or gene gun method:** Cells are bombarded with high velocity micro-particles of gold or tungsten coated with DNA in plants.

Q.8. What is *Ti* plasmid? Name the organism where it is found. How does it help in genetic engineering?

Ans. An extra-chromosomal DNA which delivers gene of interest into variety of plants and act as cloning vector is called *Ti* plasmid. They are present in *Agrobacterium tumifaciens*. Ti plasmid vectors are used for genetic transformation in many dicot plants. The tumour inducing (Ti) plasmid of *Agrobacterium tumifaciens* has 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 interest into a variety of plants.

Q.9. Expand the following and mention one application of each:

i. PCR
ii. ELISA

Ans.

		Expansion	Application
i.	PCR	Polymerase Chain Reaction	Amplification of gene of interest/In forensic study
ii.	ELISA	Enzyme Linked Immunosorbent Assay	Diagnostic test for AIDS

Q.10. Many copies of a specific gene of interest are required to study the detailed sequencing of bases in it. Name and explain the process that can help in developing large number of copies of this gene of interest.

Ans. Polymerase Chain reaction (PCR).
PCR is carried out in the following three steps:

(a) Denaturation

— The double-stranded DNA is denatured by subjecting it to high temperature of 95°C for 15 seconds. Each separated single stranded strand now acts as template for DNA synthesis.

(b) Annealing

— Two sets of primers are added which anneal to the 3′ end of each separated strand.

— Primers act as initiators of replication.

(c) Extension

— DNA polymerase extends the primers by adding nucleotides complementary to the template provided in the reaction.

— A thermostable DNA polymerase (Taq polymerase) is used in the reaction which can tolerate the high temperature of the reaction.

— All these steps are repeated many times to obtain several copies of desired DNA.

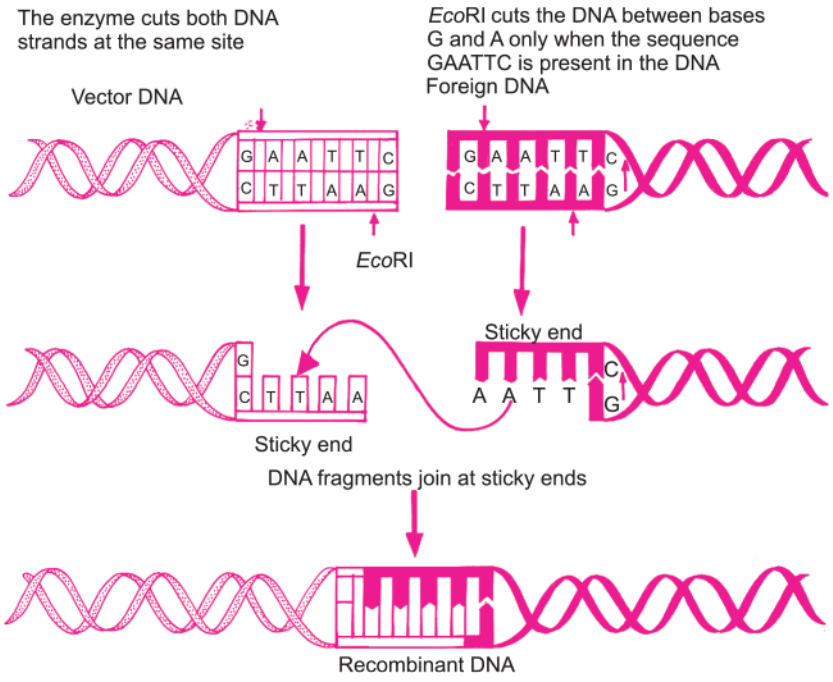
Q.11. A recombinant DNA is formed when sticky ends of vector DNA and foreign DNA join. Explain how the sticky ends are formed and get joined.

OR

***Eco*RI is used to cut a segment of foreign DNA and that of a vector DNA to form a recombinant DNA. Show with the help of schematic diagrams.**

i. The set of palindromic nucleotide sequence of base pairs the *Eco*RI will recognise in both the DNA segments. Mark the site at which *Eco*RI will act and cut both the segments.
ii. Sticky ends formed on both the segments where the two DNA segments will join later to form a recombinant DNA.

Ans. The vector DNA and foreign DNA are cut by the same restriction enzyme, such as *Eco*RI, to form the same kind of sticky ends. Then these sticky ends are joined by the enzyme DNA ligase.

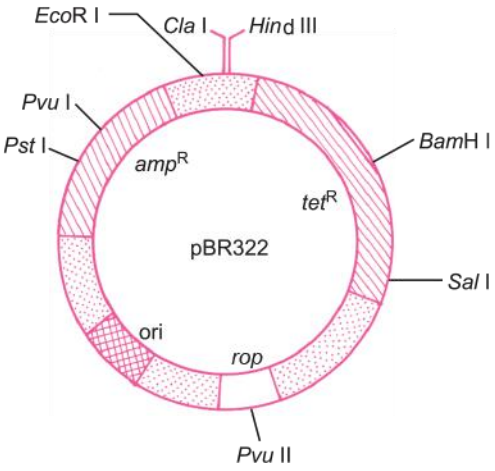


Steps in formation of recombinant DNA by action of restriction endonuclease enzyme *EcoRI*

Q.12. Draw a schematic sketch of pBR322 plasmid and label the following in it:

a. **Any two restriction sites.**
b. **ori and rop genes.**
c. **An antibiotic resistant gene.**

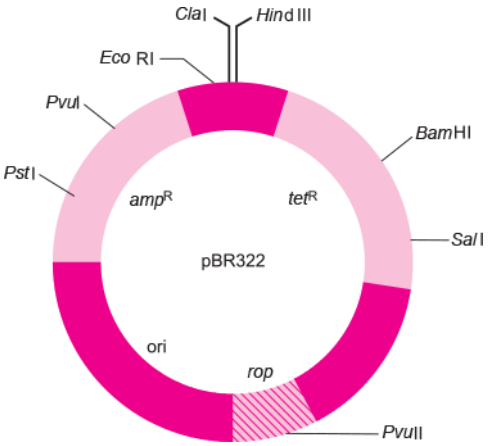
Ans.



E. coli cloning vector pBR322 showing restriction sites (HindIII, EcoRI, BamHI, SalI, PvuII, PstI, ClaI), ori and antibiotic resistance genes (amp^R and tet^R). rop codes for the proteins involved in the replication of the plasmid.

Q.13.

i. **Name the organism in which the vector shown is inserted to get the copies of the desired gene.**
ii. **Mention the area labelled in the vector responsible for controlling the copy number of the inserted gene.**
iii. **Name and explain the role of a selectable marker in the vector shown.**



Ans.

i. *Escherichia coli*.
ii. Origin of replication or '*ori*' controls copy number of inserted gene.
iii. The selectable markers are *amp*^{R} and *tet*^{R} (resistance to ampicillin, tetracycline). Selectable markers help to select the host cells which contain the vector (transformants) and eliminate non-transformants. If a foreign DNA ligates at the *Bam*HI site of tetracycline resistance gene in the vector pBR322, the recombinant plasmid loses the tetracycline resistance due to insertion of DNA. It can still be selected out from non-recombinant.

Q.14. How is the amplification of a gene sample of interest carried out using Polymerase Chain Reaction (PCR)?

OR

Suggest and describe a technique to obtain multiple copies of a gene of interest *in vitro*.

Ans. PCR is carried out in the following three steps:

(a) Denaturation

— The double-stranded DNA is denatured by subjecting it to high temperature of 95°C for 15 seconds. Each separated single stranded strand now acts as template for DNA synthesis.

(b) Annealing

— Two sets of primers are added which anneal to the 3′ end of each separated strand.

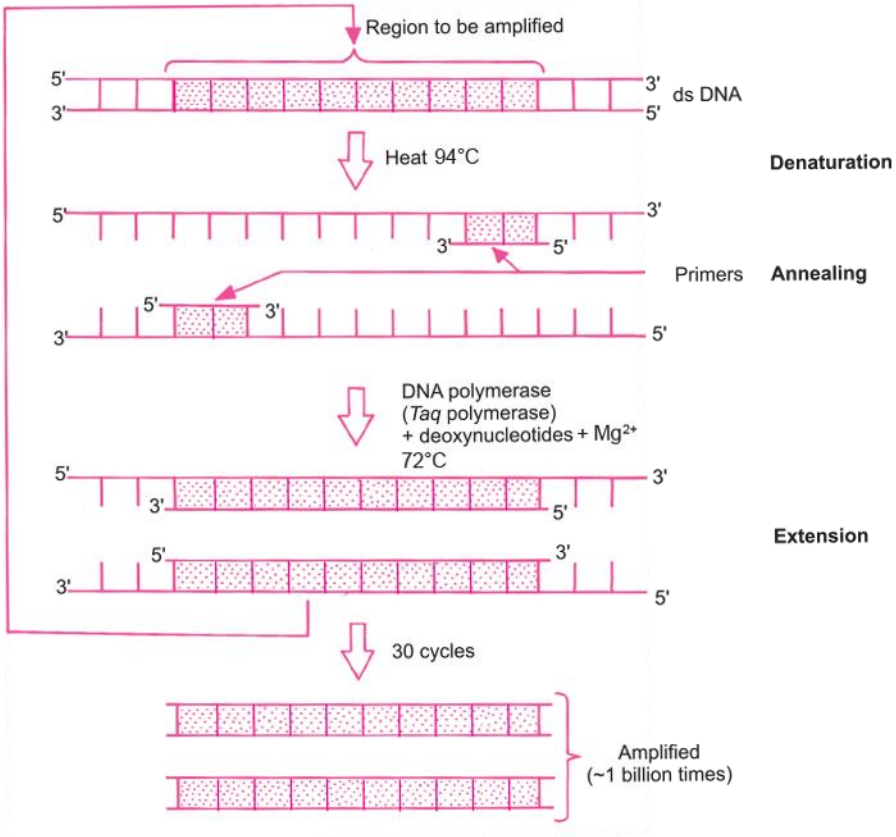
— Primers act as initiators of replication.

(c) Extension

— DNA polymerase extends the primers by adding nucleotides complementary to the template provided in the reaction.

— A thermostable DNA polymerase (Taq polymerase) is used in the reaction which can tolerate the high temperature of the reaction.

— All these steps are repeated many times to obtain several copies of desired DNA.



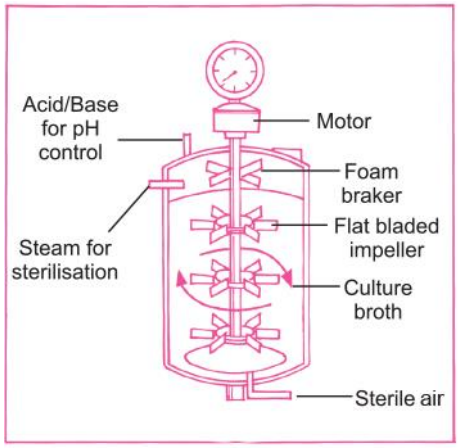
Polymerase chain reaction (PCR)

Q.15. How can a bioreactor be made to function at optimal state in order to obtain a desired foreign gene product? Explain.

Ans. A stirred-tank bioreactor is the most commonly used bioreactor. It comes with a curved base to facilitate the mixing of the reactor contents. The stirrer facilitates even mixing and oxygen availability throughout the bioreactor. The bioreactor has an agitator system, an oxygen delivery system and a foam control system, a temperature control system, pH control system and sampling port so that volumes of the cultures can be withdrawn periodically.

Q.16. Draw a labelled sketch of sparged stirred-tank bioreactor. Write its application.

Ans.



Simple stirred- tank bioreactor

Application: Produces larger biomass leading to higher yields of desired protein.

Q.17. Name two commonly used bioreactors. State the importance of using a bioreactor.

Ans. Two commonly used bioreactors are simple stirred tank bioreactor and sparged stirred tank bioreactor.
A bioreactor is used for

i. processing large volumes of culture.
ii. large scale production of recombinant proteins.
iii. biologically converting raw materials into specific products.

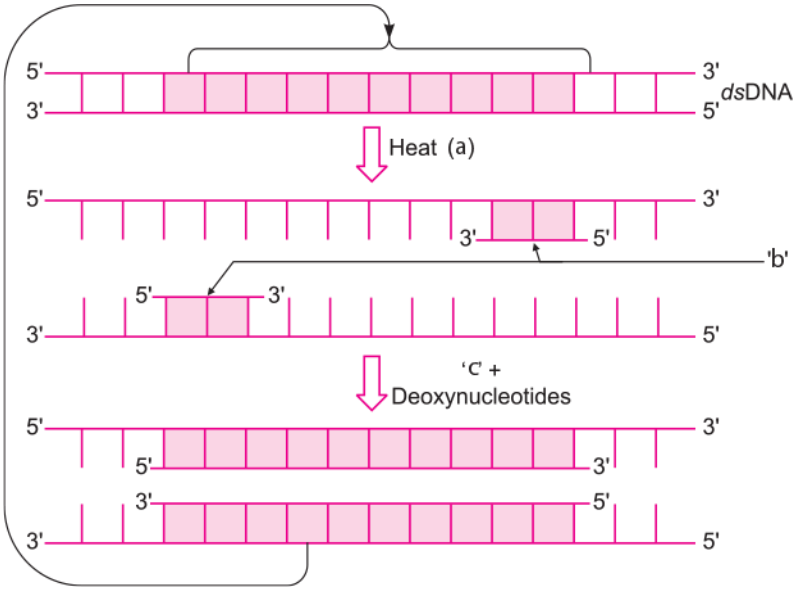
Q.18.

a. List the three steps involved in Polymerase Chain Reaction (PCR).
b. Name the source organism of *Taq* polymerase. Explain the specific role of this enzyme in PCR.

Ans.

a. The three steps involved in polymerase chain reaction (PCR):
   i. Denaturation of double stranded DNA (*ds*DNA) at high temperature.
   ii. Annealing of two sets of primers.
   iii. Extension of primers to form *ds*DNA by *Taq* polymerase and deoxynucleotides.
b. Source organism of *Taq* polymerase is the bacterium *Thermus aquaticus*. This enzyme is heat tolerant and repeatedly amplifies DNA at high temperatures.

Q.19. A schematic representation of polymerase chain reaction (PCR) up to the extension stage is given below. Answer the questions that follow:



Q. Name the process '*a*'.

Ans. *a*—Denaturation process

Q. Identify 'b'

Ans. *b*—Primers

Q. Identify '*c*' and mention its importance in PCR.

Ans. *c*—*Taq* DNA polymerase. *Taq* polymerase is a thermostable enzyme which remains activeduring the high temperature required for extension of DNA.

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

Ans. Genes encoding resistance to antibodies are considered useful selectable markers for *E. coli* cloning vector is explained using this example.

If a recombinant DNA bearing gene for resistance to an antibiotic (*e.g.*, ampicillin) is transferred into *E. coli* cells, the host cells become transformed into ampicillin-resistant cells. If these transformed cells are spread on agar plates containing ampicillin, only transformants will grow, and the non-transformed recipient cells will die as they do not contain the gene for ampicillin resistance. Thus, transformed cells can be selected. The gene for ampicillin resistance, in this case, is a useful selectable marker.

Q.21. Write the steps you would suggest to be undertaken to obtain a foreign-gene-product.

Ans. Recombinant DNA technology involves the following steps:

i. Isolation of DNA
ii. Fragmentation of DNA by restriction endonucleases.
iii. Isolation of a desired DNA fragment.
iv. Amplification of the gene of interest.
v. Ligation of the DNA fragment into a vector.
vi. Insertion of recombinant DNA into the host.
vii. Culturing the host cells on a suitable medium at a large scale.
viii. Extraction of the desired gene product.
ix. Downstream processing of the products as finished product, ready for marketing.

Q.22.

a. Explain the significance of 'palindromic nucleotide sequence' in the formation of recombinant DNA.
b. Write the use of restriction endonuclease in the above process.

Ans.

a. Palindromic nucleotide sequence is the recognition (specific) sequence present both on the vector and on a desired or alien DNA for the action of the same (specific) restriction endonuclease to act upon.
b.
    i. Every endonuclease inspects the entire DNA sequence for the palindromic recognition sequence.
    ii. On finding the palindrome, the endonuclease binds to the DNA.
    iii. It cuts the opposite strands of DNA in the sugar–phosphate backbone; a little away from the centre of the palindrome sites but between the same bases on both strands.
    iv. This results in the formation of single stranded overhanging stretches at the end of each strand called sticky ends.
    v. The sticky ends facilitate the action of the enzyme DNA ligase by readily forming hydrogen bonds with complementary strands.

Q.23. Describe the roles of heat, primers and the bacterium *Thermus aquaticus* in the process of PCR.

Ans.

- Heat denatures or helps in separation of DNA into two strands.
- Primer–Enzyme DNA Polymerase extend the primers using the nucleotides provided in the reaction and the genomic DNA as template.
- ***Thermus aquaticus:*** It is the source of thermostable DNA polymerase or *Taq* polymerase.

Q.24. Explain three basic steps to be followed during genetic modification of an organism.

Ans. The three basic steps 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 transfer of the DNA to its progeny.

Q.25. How does β-galactosidase coding sequence act as a selectable marker? Explain. Why is it a preferred selectable marker to antibiotic resistance genes?

Ans. When a recombinant DNA is inserted within the coding sequence of the enzyme β-galactosidase, it results into inactivation of the enzyme. The presence of a chromogenic substrate gives blue coloured colonies if the plasmid in the bacteria does not have an insert, whereas presence of insert do not produce any colour.

Selection of recombinants due to inactivation of antibiotics is a cumbersome procedure because it requires simultaneous plating on two plates having different antibiotics. Therefore, selectable markers are preferred for selection of recombinants.

Q.26. Suggest and describe a technique to obtain multiple copies of a gene of interest in *vitro*.

Ans. PCR is carried out in the following three steps:

(a) Denaturation

— The double-stranded DNA is denatured by subjecting it to high temperature of 95°C for 15 seconds. Each separated single stranded strand now acts as template for DNA synthesis.

(b) Annealing

— Two sets of primers are added which anneal to the 3′ end of each separated strand.

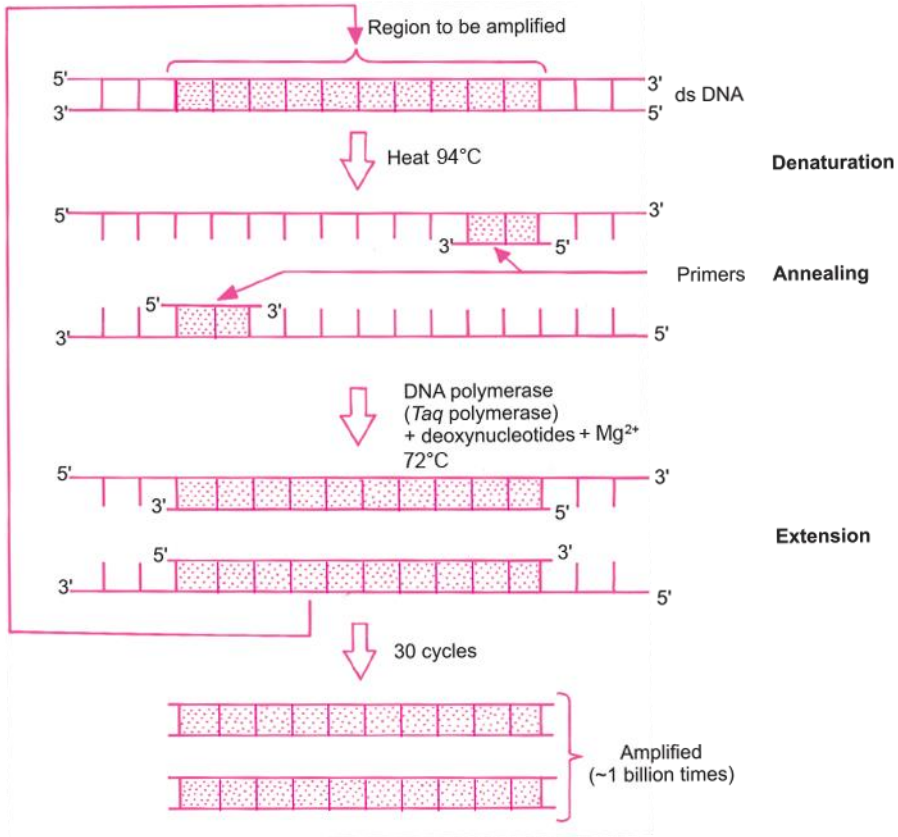
— Primers act as initiators of replication.

(c) Extension

— DNA polymerase extends the primers by adding nucleotides complementary to the template provided in the reaction.

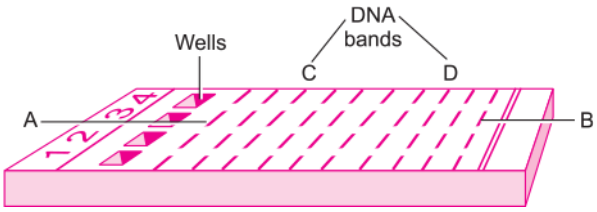
— A thermostable DNA polymerase (Taq polymerase) is used in the reaction which can tolerate the high temperature of the reaction.

— All these steps are repeated many times to obtain several copies of desired DNA.



Polymerase chain reaction (PCR)

Q.27. Study the diagram given below and answer the questions that follow:



Q. Why have DNA fragments in band 'D' moved farther away in comparison to those in band 'C'?

Ans. DNA fragments in band 'D' are smaller in size than fragments in band 'C'. Therefore, they moved faster and farther away.

Q. Identify the anode end in the diagram.

Ans. The anode end is 'B'.

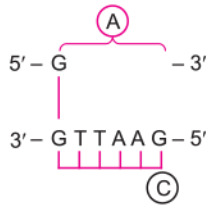
Q. How are these DNA fragments visualised?

Ans. The separated DNA fragments can be visualised by staining the DNA with ethidium bromide followed by exposure to UV radiation.

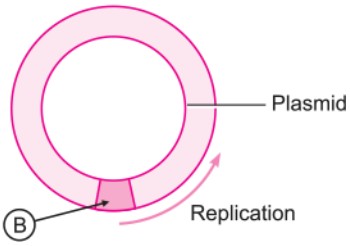
Q.28.

a. Identify Ⓐ and Ⓑ illustrations in the following:

(*i*)



(*ii*)



b. Write the term given to Ⓐ and Ⓒ , and why?
c. Expand PCR. Mention its importance in biotechnology.

Ans.

a. A—Recognition site of the restriction endonuclease.
   B—Origin of replication
b. A—Coding strand; C—Template strand.
c. PCR stands for polymerase chain reaction.
   PCR is used to amplify DNA segments to a large number within a short span of time.

Q.29. "A very small sample of tissue or even a drop of blood can help determine paternity". Provide a scientific explanation to substantiate the statement.

Ans.

i. DNA from all cells of an individual shows the same degree of polymorphism and therefore becomes a useful identification tool.
ii. Polymorphs are heritable and the child inherits 50% of the chromosome from each parent.
iii. With the help of PCR the small amount of DNA from blood can be amplified and be used in DNA finger printing to identify the paternity.

Q.30. Answer the following question:

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

Ans. A cell must be made competent so that it can take up the hydrophilic DNA from the external medium. Divalent calcium ions increase the efficiency of DNA entering the cell through pores in the cell wall.

Q. State the role of 'biolistic gun' in biotechnology experiments.

Ans. Biolistic gun is used to introduce alien DNA into the plant cell by bombarding them with high velocity microparticles (gold or tungsten coated with DNA).

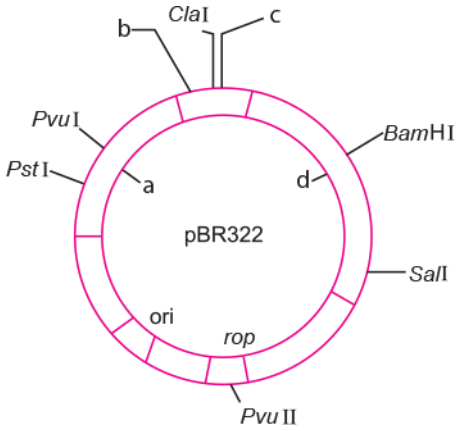
Q.31. Answer the following questons:

Q. Identify the selectable markers in the diagram of *E. coli* vector shown above.

Ans. a—gene for ampicillin resistance
d—gene for tetracycline resistance

Q.32. How is the coding sequence of β-galactosidase considered a better marker than the ones identified by you in the diagram? Explain.

Ans. The insertion of *r*DNA into the coding sequence of an enzyme β-galactosidase leads to the inactivation of the enzyme. This is called insertional inactivation. The recombinants do not produce blue-coloured colonies in the presence of chromogenic substrate while the non-recombinants produce a blue colour. Thus, coding sequence of β-galactosidase is a better marker.



Q.32. Answer the following questions:

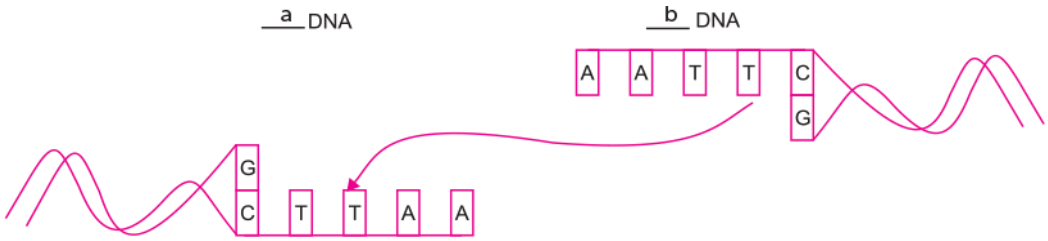
Q. Name the selectable markers in the cloning vector pBR322? Mention the role they play.

Ans. Selectable markers are *amp*^{R}/ampicillin resistance genes and tet^R/tetracycline resistance gene. They help in identifying and eliminating non-transformants/non-recombinants and selectively permitting the growth of the transformants/recombinants.

Q. Why is the coding sequence of an enzyme β-galactosidase is a preferred selectable marker in comparison to the ones named above?

Ans. This is because it is simpler and less cumbersome. In the presence of chromogenic substrate recombinants are colourless and non-recombinants are blue in colour.

Q.33.



Study the linking of DNA fragments shown above:

i. Name ‘a’ DNA and ‘b’ DNA.
ii. Name the restriction enzyme that recognises this palindrome.
iii. Name the enzyme that can link these two DNA fragments.

Ans.

i. a is vector/plasmid DNA
b is foreign DNA
ii. EcoRI recognises this palindrome
iii. DNA ligase.

Short Answer Questions-II (OIQ)

[3 Marks]

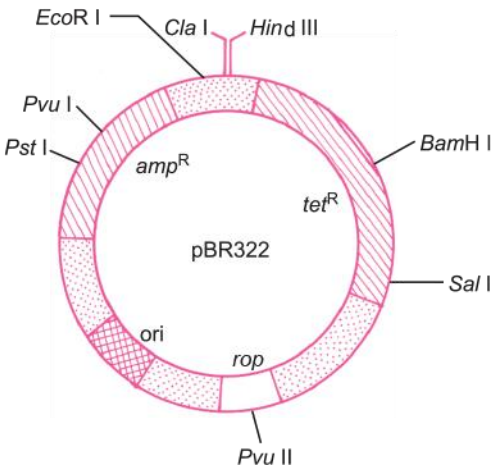
Q.1.

a. Draw the figure of vector pBR322 and label the following:
- **i. Origin of replication**
- **ii. Ampicillin resistance site**
- **iii. Tetracycline resistance site**
- **iv. *Bam*H1 restriction site**

b. Identify the significance of origin of replication.

Ans.

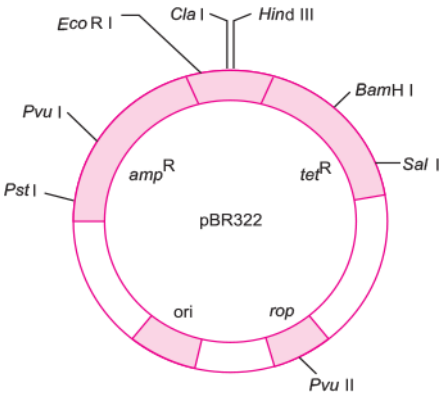
(a)



E. coli cloning vector pBR322 showing restriction sites (HindIII, EcoRI, BamHI, SalI, PvuII, PstI, ClaI), ori and antibiotic resistance genes (amp^R and tet^R). rop codes for the proteins involved in the replication of the plasmid.

(b) Origin of replication is responsible for controlling the copy number of the DNA sequence inserted.

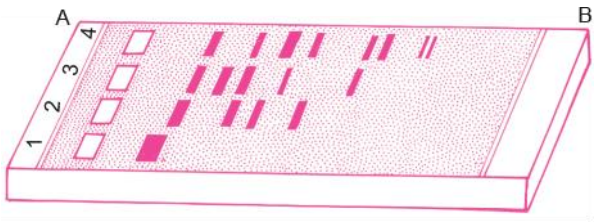
Q.2. Explain the importance of (*a*) ori, (*b*) *amp*^{R} and (*c*) *rop* in the *E. coli* vector shown below:



Ans.

a. ***ori:*** Ori is a sequence from where replication starts and any piece of DNA when linked to this sequence can be made to replicate within the host cells. It is also responsible for controlling the copy number of the linked DNA.
b. ***amp^R:*** The ligation of alien DNA is carried out at a restriction site present in any antibiotic resistance gene.
c. ***rop:*** It codes for the proteins involved in the replication of the plasmid.

Q.3.



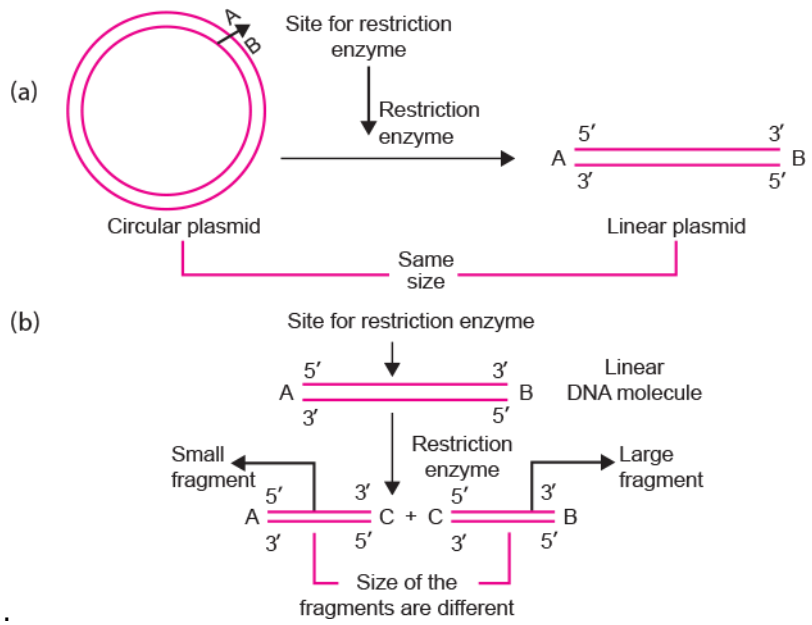
a. **Mark the positive and negative terminals.**
b. **What is the charge carried by DNA molecule and how does it help in its separation?**
c. **How the separated DNA fragments are finally isolated?**

Ans.

a. Positive terminal – 'B'
   Negative terminal – 'A'
b. DNA is negatively charged. Because of its negative charge, DNA moves towards the positive electrode (anode).
c. The separated DNA fragments are separated by elution. The separated bands of DNA are cut out from the agarose gel and extracted from the gel piece.

Q.4. A plasmid DNA and a linear DNA (both of the same size) have one site for a restriction endonuclease. When cut and separated on agarose gel electrophoresis, plasmid shows one DNA band while linear DNA shows two fragments. Explain.

Ans. It is because plasmid is a circular DNA molecule. When cut with enzyme, it becomes linear but does not get fragmented. Whereas, a linear DNA molecule gets cut into two fragments. Hence, a single DNA band is observed for plasmid while two DNA bands are observed for linear DNA in agarose gel



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Q.5. A mixture of fragmented DNA was electrophoresed in agarose gel. After staining the gel with ethidium bromide, no DNA bands were observed. What could be the reason?

Ans. The reasons that could be possible are as follows:

i. DNA sample that was loaded on the gel may have got contaminated with nuclease (exo- or endo- or both) and completely degraded.
ii. Electrodes were put in opposite orientation in the gel assembly, *i.e.*, anode towards the wells (where DNA sample is loaded). Since DNA molecules are negatively charged, they move towards anode and hence move out of the gel instead of moving into the matrix of gel.
iii. Ethidium bromide was not added at all or was not added in sufficient concentration and so DNA was not visible.
iv. After staining with Ethidium bromide it was not observed under UV

Q.6. (a) In pBR322, foreign DNA has to be introduced in tet^R region. From the restriction enzymes given below, which one should be used and why?

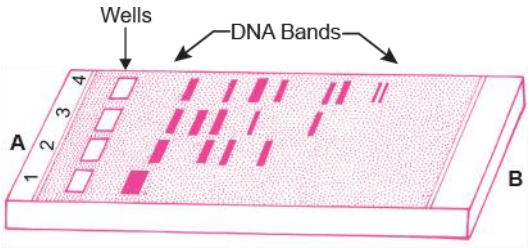
***Pvu*I, *Eco*RI, *Bam*HI**

(b) Give reasons, why the other two enzymes cannot be used.

Ans.

a. *Bam*HI should be used, as restriction site for this enzyme is present in tet^R region.
b. *Pvu*I will not be used, as restriction site for this enzyme is present in amp^R region (not in tet^R). *Eco*RI will not be used, as restriction site for this enzyme is not present in selectable marker tet^R.

Q.7. Rajesh was doing gel electrophoresis to purify DNA fragments. Given below is the sketch of the observations of the experiment performed by him.



a. At which end he would have loaded the samples and where?
b. Analyse the reason for different positions taken up by the DNA bands.
c. Elaborate the step he would have followed to visualise DNA bands.

Ans.

a. He would have loaded the samples near end A; in the wells.
b. The DNA fragments separate (resolve) according to their size through sieving effect provided by the agarose gel. Hence, the smaller the fragment size, the farther it moves.
c. After staining the DNA with ethidium bromide followed by exposure to UV radiations the DNA bands appear coloured.

Long Answer Questions (PYQ)

[5 Marks]

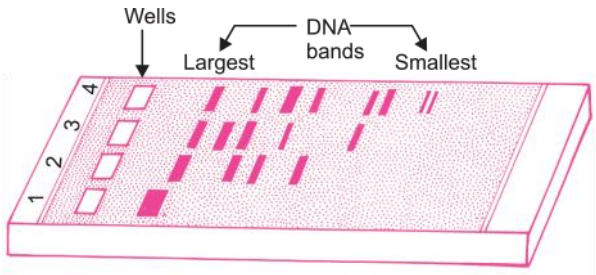
Q.1. Name and describe the technique that helps in separating the DNA fragments formed by the use of restriction endonuclease.

Ans. Gel electrophoresis helps in separating DNA fragments.

DNA fragments are negatively charged then they are forced to move towards anode under an electric field through agarose gel matrix. The fragments separate according to their size through sieving effect. Hence the smaller fragments move faster and further than the larger ones.

Separation and Isolation of DNA Fragments (Gel Electrophoresis)

- Gel electrophoresis is a technique for separating DNA fragments based on their size.
- Firstly, the sample DNA is cut into fragments by restriction endonucleases.
- The DNA fragments being negatively charged can be separated by forcing them to move towards the anode under an electric field through a medium/matrix.
- Commonly used matrix is agarose, which is a natural linear polymer of D-galactose and 3, 6-anhydro-L-galactose which is extracted from sea weeds.
- The DNA fragments separate-out (resolve) according to their size because of the sieving property of agarose gel. Hence, smaller the fragment size, the farther it will move.



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

- The separated DNA fragments are visualised after staining the DNA with ethidium bromide followed by exposure to UV radiation.
- The DNA fragments are seen as orange coloured bands.
- The separated bands of DNA are cut out and extracted from the gel piece. This step is called elution.
- The purified DNA fragments are used to form recombinant DNA which can be joined with cloning vectors.

Q.2. If a desired gene is identified in an organism for some experiments, explain the process of the following:

Q. Cutting this desired gene at specific location.

Ans. The desired gene is cut using the enzymes restriction endonucleases. Firstly, the restriction endonucleases that recognise the palindromic nucleotide sequence of the desired gene is identified. The endonuclease inspects the entire DNA sequences to find and recognise the site. It cuts each of the double helix at a specific point which is a little away from the centre of the palindromic site. The cutting site is between the same two bases on the opposite strands. This results in over-hanging single stranded stretches which act as sticky ends.

Q. Synthesis of multiple copies of this desired gene.

Ans. Multiple copies of the desired gene is synthesised by polymerase chain reaction (PCR) method. In this method, the desired gene is synthesised *in vitro*. The double stranded DNA is denatured using high temperature of 95°C and the strands are separated. Each separated strand acts as template.

Two sets of oligonucleotide primers are annealed to the denatured DNA strands. The thermostable *Taq*polymerase extends the primers, using nucleotides provided in the reaction mixture. Finally the amplified fragments are ligated into recipient cells.

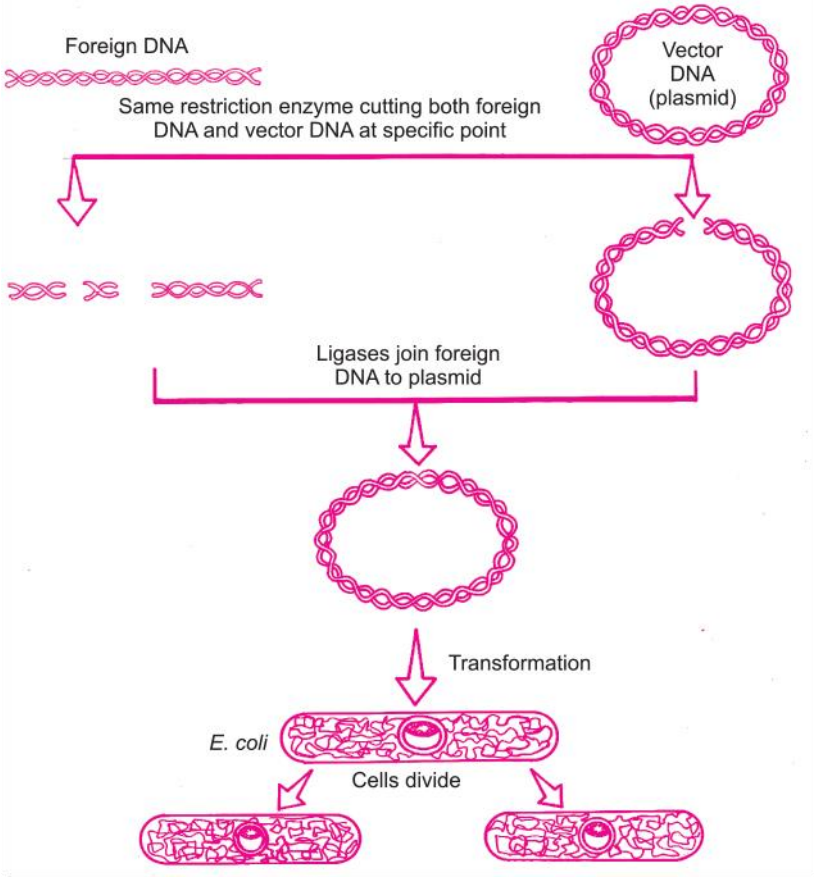
Q.3. (a) Mention the role of vectors in recombinant DNA technology. Give any two examples.

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

Ans.

a. **Role of vectors:** The vectors have the ability to replicate within the bacterial cells independent of the control of chromosomal DNA. If an alien piece of DNA is linked to the vector like bacteriophage or plasmid DNA, it can be made to multiply, its number being equal to the copy number of the vector. Vectors are also used in the selection of recombinants from nonrecombinants. Plasmids and bacteriophages are the most commonly used vectors.

b.

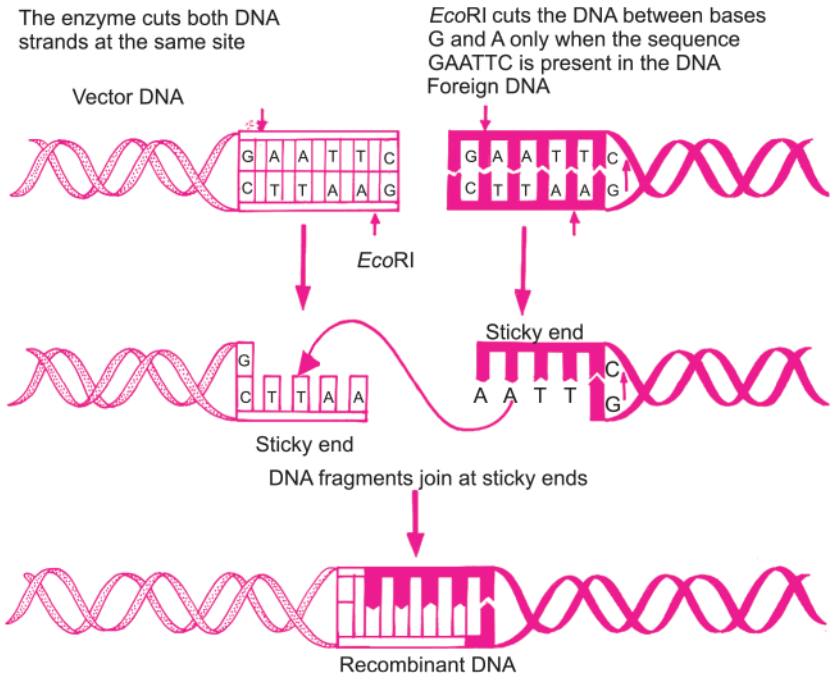


Diagrammatic representation of recombinant DNA technology

Q.4. Answer the following questions:

Q. With the help of diagrams show the different steps in the formation of recombinant DNA by action of restriction endonuclease enzyme E*coRI*.

Ans.



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

Q. Name the technique that is used for separating the fragments of DNA cut by restriction endonucleases.

Ans. Gel electrophoresis is used for separating the fragments of DNA cut by restriction endonucleases.

Q.5. Answer the following questions:

Q. Why are engineered vectors preferred by biotechnologists for transferring the desired genes into another organism?

Ans. Engineered vectors are preferred by biotechnologists because they help in easy linking of foreign DNA and selection of recombinants from non-recombinants

Q. Explain how do "*ori*", "selectable markers" and "cloning sites" facilitate cloning into a vector.

Ans.

Origin of replication (ori)

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

Selectable marker

- It helps to select the host cells which contain the vector (transformants) and eliminate the non-transformants.
- Transformation is defined as the procedure by which a piece of DNA is introduced into a bacterial host.
- Genes encoding resistance to antibiotics like ampicillin, chloramphenicol, tetracycline or kanamycin, are useful selectable markers for *E. coli.*
- The normal *E. coli* cells do not carry resistance against any of these antibiotics.

Cloning sites

- To link the alien DNA, the vectors require very few (mostly single) **recognition sites** for the restriction enzymes.
- More than one recognition sites within the vector, can complicate the gene cloning as it will generate several fragments.
- Ligation of alien DNA can be carried out at a restriction site present in one of the two antibiotic resistance genes.

Q.6. Answer the following questions:

Q. Describe the characteristics that a cloning vector must possess.

Ans. A cloning vector must have the following characteristics:

a. ori or origin of replication which can make large number of copies
b. Selectable marker, genes encoding for an antibiotic resistance or genes encoding forα-galactosidase.
c. Recognition site for the restriction enzyme to recognise.

Q. Why DNA cannot pass through the cell membrane? Explain. How is a bacterial cell made 'competent' to take up recombinant DNA from the medium?

Ans. DNA is a hydrophilic molecule, therefore it cannot pass through the cell membrane.

The bacterial cells can be made competent by treating them with a specific concentration of a divalent ion like calcium. The cells are then incubated on ice followed by a heat shock by placing them briefly at 42°C and then putting back on ice.

Long Answer Questions (OIQ)

[5 Marks]

Q.1. What is *r*DNA? Explain the technique of cloning *r*DNA.

Ans. *r*DNA is the DNA formed by combining DNAs from two different organisms. In bacterial cell, *r*DNA can be transferred by using a vector where it multiplies into many copies. These copies are preserved as gene library.
Recombinant DNA technology involves the following steps:

i. Isolation of DNA
ii. Fragmentation of DNA by restriction endonucleases.
iii. Isolation of a desired DNA fragment.
iv. Amplification of the gene of interest.
v. Ligation of the DNA fragment into a vector.
vi. Insertion of recombinant DNA into the host.
vii. Culturing the host cells on a suitable medium at a large scale.
viii. Extraction of the desired gene product.
ix. Downstream processing of the products as finished product, ready for marketing.

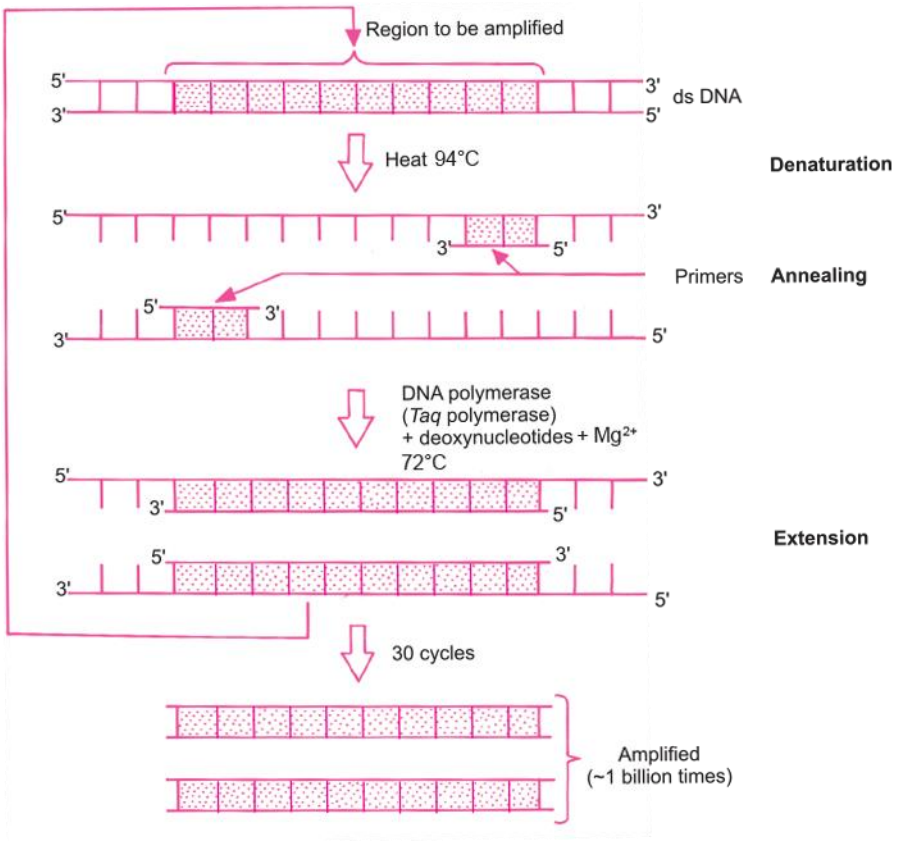
Q.2. Which methodology is used while sequencing the total DNA from a cell? Explain it in detail.

Ans. Methodology used:

- Sequence Annotation – total DNA from a cell is isolated, converted into random fragments of relatively smaller sizes, and cloned in suitable host using specialized vectors.
- The cloning resulted into amplification of each piece of DNA fragment.
- The fragments were sequenced using automated DNA sequencers, these sequences are then arranged based on some overlapping regions (present in them).
- This requires generation of overlapping fragments (for sequencing).
- Specialised computer based programmes were developed, and these sequences were subsequently annotated and assigned to each chromosome.

Q.3. Represent diagrammatically the steps in amplification of a DNA segment. Who designed the process?

Ans.



Polymerase chain reaction (PCR)

This process was designed by **K. Mullis**.

Q.4. Explain the different steps in the construction of recombinant DNA.

Ans.

Isolation of the genetic material (DNA)

- RNA is removed by treatment with ribonuclease and proteins are removed by treatment with protease.
- After several treatments, the purified DNA is precipitated by adding chilled ethanol.
- The bacterial/plant/animal cell is broken down by enzymes to release DNA, along with RNA, proteins, polysaccharides and lipids.
- Bacterial cell is treated with enzyme lysozyme.
- Plant cell is treated with enzyme cellulase.
- Fungal cell is treated with chitinase.

Cutting of DNA at specific locations

- The DNA is cut using restriction enzymes.

- The purified DNA is incubated, with the specific restriction enzyme at conditions optimum for the enzyme to act.

Isolation of desired DNA fragment

- Using agarose gel electrophoresis, the activity of the restriction enzymes can be checked.
- Since the DNA is negatively charged, it moves towards the positive electrode or anode and in the process, DNA fragments separate out based on their sizes.
- The desired DNA fragment is eluted out.

Amplification of gene of interest using PCR

- The Polymerase Chain Reaction (PCR) is a reaction in which amplification of specific DNA sequences is carried out in vitro.

Q.5. For selection of recombinants, insertional inactivation of antibiotic marker has been superceded by insertional inactivation of a marker gene coding for a chromogenic substrate. Give reasons.

Ans. Selection of recombinants due to inactivation of antibiotics is a laborious process as it requires:

i. a vector with two antibiotic resistance markers,
ii. preparation of two kinds of media plates, with one antibiotic each.

Transformed cells are first plated on the antibiotic plate which has not been insertionally inactivated (say, ampicillin) and incubated overnight for growth of transformants. For selection of recombinants, these transformants are replica-plated on second antibiotic (say, tetracycline) plate (which got inactivated due to insertion of gene). Non-recombinants grow on both the plates (one carrying ampicillin and the other carrying tetracycline) while recombinants will grow only on ampicillin plate.

This entire exercise is laborious and takes more time (two overnight incubation) as well. However, if we choose insertional inactivation of a marker that produces colour in the presence of a chromogenic compound, we can distinguish between the recombinants and non-recombinants on a single medium plate (containing one antibiotic and the chromogenic compound) after overnight growth.

Q.6. Answer the following questions:

Q. Explain how recombinants and non-recombinants are differentiated on the basis of colour production in the presence of a chromogenic substrate. Name that procedure.

Ans. The procedure is called insertional inactivation.

In this method recombinants and non-recombinants are differentiated on the basis of the ability to produce colour in the presence of a chromosomic substrate. In this method, a *r*DNA is inserted in an enzyme – β-galactosidase which leads to inactivation of the enzyme which does not produce colour due to insertion.

Q. Describe the temperature treatment that enhances the bacteria to take up the *r*DNA.

Ans.

i. Host cells are incubated with *r*DNA on ice.
ii. Followed by placing them briefly at 42°C.
iii. Then transfer them back on ice.

This enables the host cells (bacteria) to take up the *r*DNA.