CHAPTER 11

BIOTECHNOLOGY

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.
2. 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.
3. 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.
4. Suggest a technique to a researcher who needs to separate fragments of DNA.
   Ans. Gel electrophoresis is used to separate DNA fragments.
5. 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.
6. 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.
7. 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.
8. 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 called sticky ends.

9. 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., EcoRI. (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.

10. Explain the role of the enzyme EcoRI in recombinant DNA technology.
Ans. EcoRI 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. Refer to Basic Concepts Point 4 (Mechanism of Action of Endonucleases). The first endonuclease discovered was HindII.

11. 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

12. 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.

13. 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, EcoRI, BamHI.

14. .(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.

15. 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.

16. 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 does 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.

17. 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.

18. 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.
19. 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.
20. (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 nontransformants, 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.
21. 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
22. 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 of linked DNA. If we want to produce many copies of target DNA, we should clone in a vector whose ori supports high copy number. 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 a-galactosidase.
23. Name the most commonly used bioreactor and describe its working.
    Ans. The most commonly used bioreactor is stirred-tank bioreactor. A stirred-tank bioreactor is usually cylindrical and have a stirrer which mixes the reactor contents evenly and makes oxygen available throughout the bioreactor. Optimum conditions of temperature, pH and foam control are provided.
24. 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.
25. (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.

26. Explain the action of the restriction endonuclease EcoRI.
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, a 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.

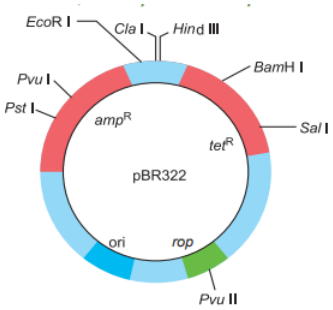
27. (a) Name the selectable markers in the cloning vector pBR322? Mention the role they play. (b) Why is the coding sequence of an enzyme β-galactosidase is a preferred selectable marker in comparison to the ones named above?
Ans. (a) Selectable markers are ampR/ampicillin resistance genes and tetR/tetracycline resistance gene. They help in identifying and eliminating non-transformants/non-recombinants and selectively permitting the growth of the transformants/recombinants. (b) This is because it is simpler and less cumbersome. In the presence of chromogenic substrate recombinants form colourless colonies and non-recombinants form blue in colonies.

28. (a) In pBR322, foreign DNA has to be introduced in tetR region. From the restriction enzymes given below, which one should be used and why? PvuI, EcoRI, BamHI (b) Give reasons, why the other two enzymes cannot be used.
Ans. (a) BamHI should be used, as restriction site for this enzyme is present in tetR region. (b) PvuI will not be used, as restriction site for this enzyme is present in ampR region (not in tetR). EcoRI will not be used, as restriction site for this enzyme is not present in selectable marker tetR.

29. 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.

30. Explain the importance of (a) ori, (b) ampR 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) ampR: 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.

31. (a) Why must a cell be made 'competent' in biotechnology experiments? How does calcium ion help in doing so? (b) State the role of 'biolistic gun' in biotechnology experiments.

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

32. 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.

33. 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.

34. (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 (dsDNA) at high temperature. (ii) Annealing of two sets of primers. (iii) Extension of primers to form dsDNA by Taq polymerase and deoxynucleotides. (b) Source organism of Taq polymerase is the bacterium

Thermus aquaticus. This enzyme is heat tolerant and can repeatedly amplify DNA at high temperatures.

35. (i) Describe the characteristics that a cloning vector must possess. (ii) 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. (i) A cloning vector must have the following characteristics: (a) ori or origin of replication which can make large number of copies (b) Selectable marker i.e., genes encoding for an antibiotic resistance or genes encoding for α-galactosidase. (c) Recognition site for the restriction enzyme to recognise. (ii) 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.

36. If a desired gene is identified in an organism for some experiments, explain the process of the following: (i) Cutting this desired gene at specific location. (ii) Synthesis of multiple copies of this desired gene.
Ans. (i) 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. (ii) 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.

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