Chapter 10.Biotechnology: Principles and Processes

Principles of Biotechnology and Tools of Recombination DNA Technology

1 Mark Questions

1. Why is it not possible for an alien DNA to become part of a chromosome anywhere along its length and replicate normally?
[All India 2014]

Ans. The integration of alien DNA is required to become a part of chromosome. As the DNA itself cannot multiply and replicate but rather requires a specific sequence for initiating its replication called origin of replication. Therefore, the alien DNA needs to be joined with the host DNA with the help of enzymes and linked to Ori, so as to be part of chromosome and replicate normally to produce its copies.

2. Mention the type of host cells suitable for the gene guns to introduce an alien DNA. [Delhi 2014]

Ans. The host cells suitable for the gene guns to introduce an alien DNA are plant cells.

3. Write the two components of first artificial recombinant DNA molecule constructed by Cohen and Boyer. [Foreign 2014]

Ans. The two components of first artificial recombinant DNA molecule constructed by Gohen and Boyer are:
(i) antibiotic resistance gene
(ii) plasmid of Salmonella typhimurium

4. Name the host cells in which microinjection technique is used to introduce an alien DNA. [Foreign 2014]

Ans. The microinjection technique to introduce alien DNA is usually carried out in animal cell, i.e. directly into the nucleus.

5. Name the material used as matrix in gel electrophoresis and mention its role. [All India 2014C]

Ans. The material used as matrix in gel electrophoresis is agarose.
This agarose gel acts as a sieve to separate the DNA fragments according to their size.

6. Write any four ways used to introduce a desired DNA segment into a bacterial cell in recombinant technology experiments. [All India 2013]

Ans. Ways to introduce desired DNA into bacterial cell are:
(i) microinjection (ii) disarmed pathogen vectors
(iii) portion by bivalent cation such as calcium
(iv) bidlistic or gene gun

7. State what happens when an alien gene is ligated at Sal I site of pBR322 plasmid. [Delhi 2013c]

Ans. If an alien gene is ligated at Sal I site of tetracycline resistance gene in the vector pBR322, the recombinant plasmid will lose its tetracycline resistance.

8. Mention the uses of cloning vector in biotechnology ? [Delhi 2011]

Ans. Uses of cloning vector in biotechnology.
(i) Helps in linking the foreign/alien DNA with that of host's DNA.
(ii) Help in the selection erf recombinants from the non-recombinants.

9. Biotechnologists refer to Agrobacterium tumefaciens as natural genetic engineer of plants. Give reasons to support the statement.
[HOTS; All India 2011]

Ans. Agrobacterium tumefacieps is a pathogen of several dicot plants. It is used as a natural genetic engineer because it is able to deliver a piece of its DNA (called T-DNA) to transform normal plant cells into tumour cells and direct the tumour cells to synthesise the chemicals required by the pathogen.

10. Why is it essential to have a selectable marker in a cloning vector? [All India 2011]

Ans. Selectable marker in cloning vector helps in identifying and selecting the recombinants and eliminating the non-recombinants.

11. Why do DNA fragments move towards the anode during gel electrophoresis? [hots; Delhi 2011c]

Ans. DNA fragments are negatively charged molecules and hence, moves toward the anode during gel electrophoresis.

12. In the year 1963, two enzymes responsible for restricting the growth of bacteriophage in E. coli were isolated. How did enzymes act to restrict the growth of the bacteriophage? [All India 2011c]

Ans. Two enzymes responsible for restricting the growth of bacteriophage in coli are: Exonucleases Add methyl group to DNA. Endonucleases Cut DNA at specific points.

13. How is the action of exonuclease different from that of endonuclease.
[All India 2010]

Ans. Exonuclease removes nucleotides from theends of DNA, while endonuclease cuts the DNA at specific positions.

14. Mention the role of molecular scissors in recombinant DNA technology. [All India 2009]

Ans. Molecular scissors or restriction enzymes cut DNA at specific site, thus allowing to extract desired gene and like it with DNA of host.

15. Name the technique used for separating DNA fragments in the laboratory. [Delhi 2008]

Ans. Gel electrophoresis is used for separating DNA fragments in the lab.

16. What is the role of ethidium bromide during agarose gel electrophoresis of DNA fragments? [All India 2008C]

Ans. The separated DNA fragments during agarose gel electrophoresis are visualised after staining the DNA with ethidium bromide, in UV light. This staining imparts DNA a bright orange colour.

2 Marks Questions

17. How does a restriction nuclease function? Explain. [All India 2014]

Ans. Restriction nucleases function by inspecting the length of DNA sequence, and then binding to specific recognition sequences and cutting the strands at sugar phosphate backbones.
These nucleases are of two types depending on their mode of action.
(i) **Restriction exonucleases** cut sequences at terminal ends of DNA.
(ii) **Restriction endonucleases** cut between the two bases of recognition sequence.

18. Write the role of Ori and restriction site in a cloning vector pBR322.
[Delhi 2014]
or
How do Ori and cloning sites facilitate cloning into a vector?
[All India 2008C]

Ans. Ori is a sequence of DNA from where replication starts. Any piece of DNA that needs to replicate in the host cell has to be linked to it.
Cloning sites refers to the site/sequence of DNA where the alien DNA is linked.

19. Explain with the help of a suitable example the naming of a restriction endonuclease. [Delhi 2014]

Ans. Naming of restriction endonuclease are:
(i) The first letter of the name comes from the genus and next two letters from species of the prokaryotic cell from where enzymes are extracted.
(ii) The Roman numbers following the name show the order in which the enzymes, were isolated from the bacterial strain. For example, Eco Rl is derived from Escherichia coli RY 13, Hind II from Haemophilus influenzae Rd, etc.

20. How are sticky ends formed on a DNA strand? Why are they so called? [Delhi 2014]

Ans. Sticky ends on DNA are formed by action of enzymes restriction endonucleases. These enzymes cut the strand of DNA a little away from the centre of the palindrome sequence between the same two bases on both the strands. This results in single stranded stretches on both the complementary strands at their ends.
These overhanging stretches are called sticky ends as they form hydrogen bonds with the complementary base pair sequences.

21.How is insertional inactivation of an enzyme used as a selectable marker to differentiate recombinants from non-recombinants?
[Foreign 2014]

Ans. The insertional activation of J3-galactosidase enzyme, i.e. by inserting the desired gene in the coding region of enzyme, results in inactivation of (3-galactosidase gene in recombinants. The recombinant on transformed hosts are unable to produce any colour when grown on chromogenic

substrate, thus acting as a selectable marker to differentiate recombinants from non-recombinants.

22. Explain palindromic nucleotide sequence with the help of a suitable example. [Foreign 2014]

Ans. The palindromic nucleotide sequence is the sequence of base pairs in DNA that reads the same on both the complementary strands of DNA, with same orientation of reading.
For example
5′-GAATTC-3′
3′-CTTAAG-5′

23. Why are molecular scissors so called? Write their use in biotechnology? [Foreign 2014]

Ans. Molecular scissors are so called because they cut DNA at specific sequences between base pairs.
Since, molecular scissors on restriction enzymes cut DNA at desired sequences and generate sticky ends that facilitate to join with host genome or vector DNA, they play an important role in genetic engineering or biotechnology. It is because with the help of these enzymes we can cut the desired gene and introduce into vectors for expression.

24. Why is making cells competent essential for biotechnology experiments? List any two ways by which this can be achieved.
[Delhi 2014C]

Ans. Since, DNA molecules are hydrophillic, they cannot pass through cell membranes. For recombinant DNA to be integrated into vector or host genome it is necessary for the DNA to be inserted in the cell. Therefore, making the host cells competent is necessary in biotechnology experiments.
The two ways by which cells can be made competent to take up DNA are:
(i) Chemical action By increasing concentration of divalent cation, calcium, thereby increasing the efficiency of DNA entering through pores in cell, wall.
(ii) Heat shock treatment Incubating the cells with recombinant DNA on ice, followed by brief treatment of heat at 42 °C and again putting them back on ice.

25. How is an exonuclease functionally different from an endonuclease? Give an example of any two endonucleases other than Sal I. [Delhi 2013C]

Ans. Exonucleases are the enzymes which cleaves base pairs of DNA at their terminal ends and act on single strand of DNA or gaps in double stranded DNA. While, endonucleases cleaves DNA at any point except the terminal ends and can make cut on one strand or on both strands of double stranded DNA, e.g. Eco Rl and Hind II.

26. Explain the work carried out by Cohen and Boyer that contributed immensely in biotechnology. [Delhi 2012]

Ans. (i) Stanley Cohen and Herbert Boyer constructed the first artificial recombinant DNA (rDNA) molecule.
(ii) They isolated the antibiotic-resistance gene by cutting out a piece of DNA from a plasmid with the help of restriction enzyme and linked it to a native plasmid of Salmonella typhimurium with the help of DNAIigase.

27. (i) A recombinant vector with a gene of interest inserted within the gene of a-galactosidase enzyme is introduced into a' bacterium. Explain the method that would help in selection of recombinant colonies from non-recombinant colonies.
(ii) Why is this method (.of selection referred to as insertional inactivation? [HOTS; All India 2012]

Ans. (i) The recombinant colonies can be differentiated from non-recombinant colonies by their inability to produce colour in the presence of a chromogenic substrate.
The recombinants do not produce any colour while, the non-recombinants produce a blue colour with chromogenic substrate in the medium.
(ii) The enzyme a-galactosidase become inactivated on insertion of recombinant DNA, within the coding sequence of enzyme. Thus, the method is called insertional inactivation.

28. Explain giving reasons why an alien piece of DNA needs to be integrated to a specific sequence of host DNA for its cloning?
[All India 2011]

Ans. The replication of DNA is initiated from the specific DNA sequence called origin of replication. For multiplication of alien DNA in the host, it has to be integrated to the origin of replication (ori).

29. List the key tools used in recombinant DNA technology. [Delhi 2011]

Ans. Key tools used in recombinant technology are restriction enzymes, polymerases, ligases, cloning vectors.and competent host organism or cells.

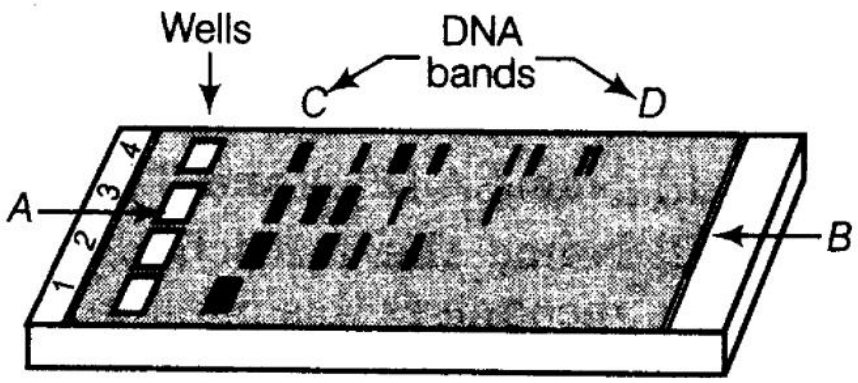
30. Explain the role of Ti plasmids in biotechnology. [Delhi 2011]

Ans. The Ti plasmid of Agrobacterium is responsible for the natural transformation of plant cells into tumours. So, it is modified into a non-pathogen ic vector but still is able to deliver the DNA. This disarmed plasmid of Agrobacterium is used as a vector for the transformation of plant cells, thus proved to play an important role in biotechnology.

31. How are recombinant vectors created? Why is only one type of restriction endonuclease required for creating one recombinant vector? [Foreign 2011]

Ans. Creation of recombinant vectors Vector DNA is cut at a particular restriction site by a restriction enzyme, the same that was used to cut the desired DNA segment. The alien DNA is then linked with the vector DNA using enzyme ligase' to form the recombinant vector.
Since, a restriction enzyme Recognises and cuts the DNA at a particular sequence called recognition site, the same restriction enzyme is used for cutting the DNA segment from both the vector and the other source, sp as to produce same sticky ends in both DNA molecules to facilitate their joining.

32. Study the diagram given below and answer the following questions



(i) Why have DNA fragments in bank D moved farther away in comparision to those in band C ?
(ii) Identify the anode end in digram.
(iii) How are these DNA fragments visualised ? [Foreign 2011]

Ans. (i) In band D, DNA fragments are smaller than those on band C. The fragments separate according to their size through the sieving effect provided by the gel So, the smaller fragments move farther away than the larger ones.
(ii) B is anode end in the diagram.
(iii) Gel containing DNA fragments is stained with ethidium bromide and exposed to UV radiation. Orange colour bands of DNA becomes visible.

33. 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 ? [All India 2010]

Ans. Sticky ends are formed when a restriction enzyme cuts the strands of DNA a little away from the centre of the palindromic sequence.
The sticky ends are joined via complementary factor of two polynucleotide strands bases and by the action of enzyme, DNA ligase.

34. Explain the action of the restriction endonuclease Eco RI.
[Foreign 2010]

Ans. Restriction endonuclease Eco Rl cuts the DNA strands a little away from the centre of the palindromic sequence, but between the same two bases on the two strands, i.e. G and A.
5′-GAATTC-3′
3′- C T T A A G- 5′
(i) Due to this, single stranded portions called sticky ends, overhang at the end of each strand.
(ii) Because of the stickiness, they easily form hydrogen bonds with their complementary counterparts.

35. How are the DNA fragments separated by gel electrophoresis visualised and separated for use in constructing recombinant DNA ?
[Foreign 201; All India 2008]

Ans. The separated DNA fragments are stained with ethidium bromide.
(i) By the exposure to UV radiation, the separated DNA fragments become visible as orange-coloured bands.

(ii) The separated bands of DNA are cut out from the agarose gel and DNA is extracted from these gel pieces, this process is called elution.

36. (i) Illustrate the recognition sequence of Eco RI and mention what such sequences are called?
(ii) How does restriction endonuclease act on a DNA molecule?
[All India 2010 C]

Ans.(/') Recognition sequence of Eco Rl
5'- G A A T T C -3'
3'- C T T A A G -3'
These sequences are called palindromic nucleotide sequences.
(ii) Restriction endonuclease acts on specified length of a DNA and binds to the DNA at the specific recognition sequence. It cuts both the double strands of DNA, at the sugar phosphate backbones, a little away from the centre of palindromic sites in between the specific sequence or points.

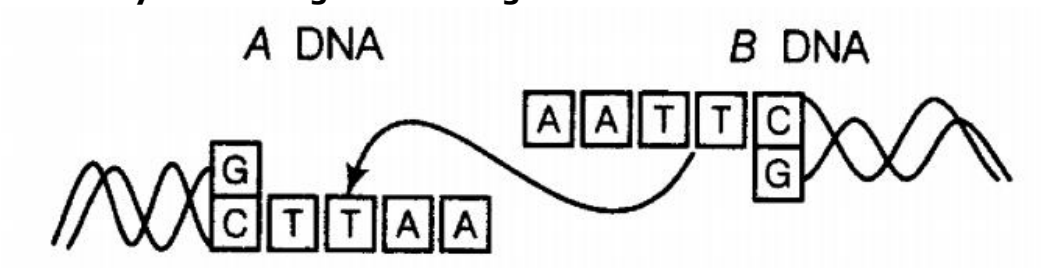
37. Name the source organism from which Ti plasmid is isolated. Explain the use of this plasmid in biotechnology. [Foreign 2009]

Ans. Ti plasmid is isolated from Agrobacterium tumefaciens bacteria.
The Ti plasmid of Agrobacterium is responsible for the natural transformation of plant cells into tumours. So, it is modified into a non-pathogen ic vector but still is able to deliver the DNA. This disarmed plasmid of Agrobacterium is used as a vector for the transformation of plant cells, thus proved to play an important role in biotechnology.

38. Name the natural source of agarose. Mention one role of agarose in biotechnology. [Delhi 2009c]

Ans. The natural source of agarose is sea weed. Role of agarose in biotechnology
(i) It is used to develop the matrix for gel electrophoresis.
(ii) It helps in the separation of fragments on the basis of their size.

39. Study the linking of DNA fragments shown below:



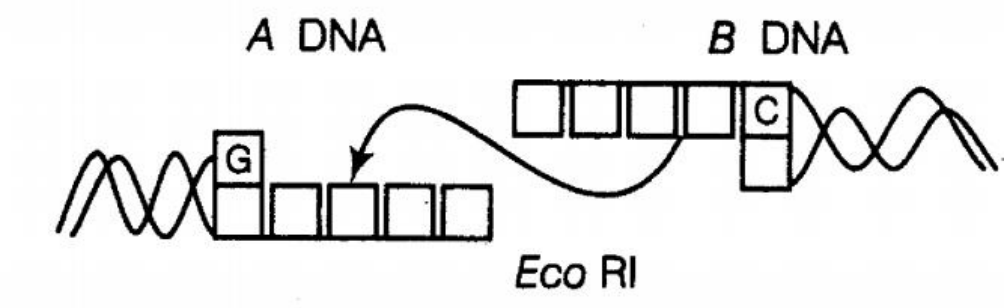
E.coli cloning vector pBR322 showing restriction sites (Hind III, Eco Rl
Bam HI, Sal I, Pvu II, Pst I, Cla I), ori and antibiotic resistance genes (amp^R and tet^R).
rop codes for the proteins involved in the replication of the plasmid.

- **Name A DNA and B DNA.**
- **Name the restriction enzyme that recognises this palindrome.**

Name the enzyme that can link these two DNA fragments.
[Delhi 2008]

Ans. (i) A – Vector/plasmid DNA
B – Foreign DNA
(ii) Eco Rl

(iii) DNA ligase**40. The following illustrates the linking of DNA fragments.**



(i) Name A and B.
(ii) Complete the palindrome, which is recognised by Eco RI.
(iii) Name the enzyme that can link the two DNA fragments.
[Foreign 2008]

Ans.(i) A – Vector DNA, B-Foreign DNA
(ii) 5′- GAATTC-3′
3′- CTTAAG-5′
(iii) DNA ligase

3 Marks Questions

41. Name and describe the technique that helps in separating the DNA fragments formed by the use of restriction endonuclease. [All India 2014]

Ans. DNA fragments formed by the use of restriction endonucleases are separated by gel electrophoresis.
(i) DNA fragments are negatively charged molecules. Thus, they move towards the anode under electric field through the medium.
(ii) DNA fragments separate according to their size due to seiving effect of agarose gel.
(iii) The separated DNA fragments can be viewed by staining the DNA with ethidium bromide followed by exposure to UV radiation.
(iv) The separated bands of DNA are cut and extracted from gel piece. This is known as elution.

42. Draw a schematic diagram of the colicloning vector pBR322 and mark the following in it.

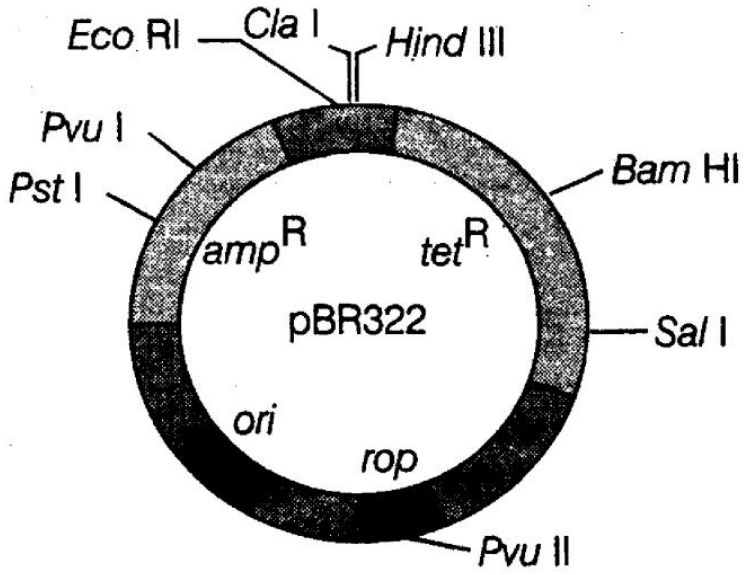
- **ori**
- **rop**
- **ampicillin resistance gene**
- **tetracycline resistance gene**
- **restriction site Bam HI**
- **restriction site EcoRI**

or

(i) Draw schematic diagrams of segments of a vector and a foreign DNA with the sequence of nucleotides recognised by Eco
(ii) Draw the vector DNA segment arid foreign DNA segments after the action of EcoRI and label the sticky ends produced. [Delhi 2014C]
or
Draw a schematic sketch of pBR322 plasmid and label the following in it

- **Any two restriction sites.**
- **Ori and rop**
- **An antibiotic resistant gene. [Delhi 2012].**

Ans. coli cloning vector pBR322.



E.coli cloning vector pBR322 showing restriction sites (Hind III, Eco RI
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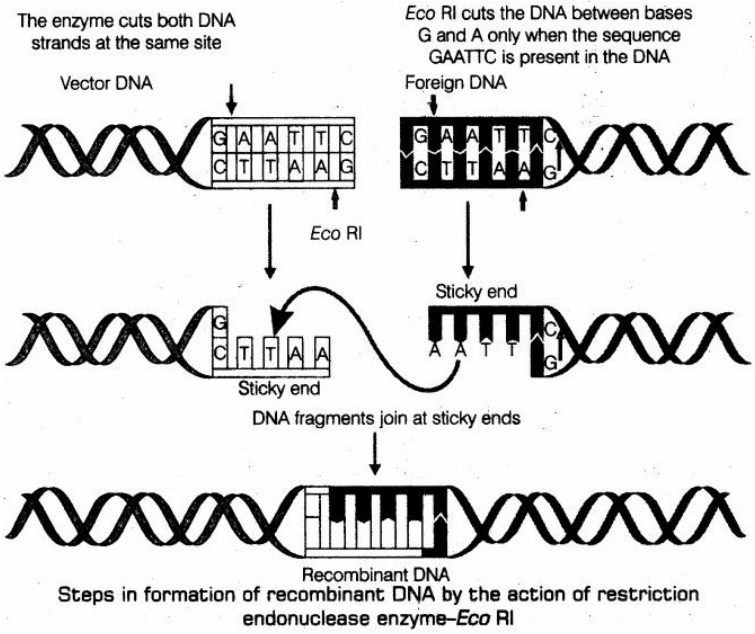
or



(a) Restriction enzymes cut the strand of DNA a little away from the centre of the palindrome sites, but between the same two bases on the opposite strands.
(b) This leaves single stranded portions at the ends.
(c) There are overhanging stretches called sticky ends on each strands as given in above figure. These are named so, because they form hydrogen bonds with their complementary cut counterparts.

(d) The stickiness of the ends facilitates the action of the enzyme DNA ligase.
(e) Restriction endonucleases are used in genetic engineering to form recombinant molecules of DNA, which are composed of DNA from different sources/genomes.
(f) These sticky ends are complementary to each other when cut by same restriction enzyme, therefore can be joined together (end-to-end) using DNA ligases.**43. What are 'cloning**

43. sites' in a cloning vector? Explain their role. Name any two such sites in pBR322. [All India 2014C]

Ans. The cloning sites are actually the specific unique recognition sequence for a particular restriction enzyme, so as to link the foreign DNA with the vector DNA to create a recombinant DNA molecules, (l) These sites are important for joining of DNA fragments of vector and alien DNA. And also multiple recognition sequences for a particular restriction enzyme within a DNA or vector will complicate the process of gene cloning.
The two cloning sites in pBR322 are Bam HI of tetracycline resistant gene and Pvu I of ampiciMies resistant genes.

44. How are the DNA fragments separated and isolated for DNA fingerprinting? Explain. [Foreign 2012]

Ans. DNA fragments formed by the use of restriction endonucleases are separated by gel electrophoresis.
(i) DNA fragments are negatively charged molecules. Thus, they move towards the anode under electric field through the medium.
(ii) DNA fragments separate according to their size due to seiving effect of agarose gel.
(iii) The separated DNA fragments can be viewed by staining the DNA with ethidium bromide followed by exposure to UV radiation.
(iv) The separated bands of DNA are cut and extracted from gel piece. This is known as elution

45. (i) Why are restriction endonucleases, so called?
(ii) What is a palindromic , nucleotide sequence? How do restriction endonucleases act on palindromic sites, to create sticky ends?
[Delhi 2011C]

Ans. (i) Restriction endonucleases are so called because they recognise and make a cut at specific positions within the DNA and restrict the growth of bacteriophage.
(ii) (a) The palindrome in DNA is a sequence of base pairs that reads same on the two strands of DNA when orientation of reading is kept the same.
For example,
5'—GAATTG—3'
3'—CTTAAG—5'
(b) Restriction enzymes cut the strand of DNA a little away from the centre of palindrome site, but between the same two bases in both the strands. This creates single stranded stretches, overhanging at the ends of the palindrome, called sticky ends.

46. How are the following used in biotechnology?

- **Plasmid DNA**
- **Recognition sequence**
- **Gel electrophoresis [All India 2011c]**

Ans. (i) Plasmid DNA It is used for constructing recombinant DNA, by ligating the alien piece of DNA with it. It is used as a cloning vector and helps in the selection of recombinants from non-

recombinants.

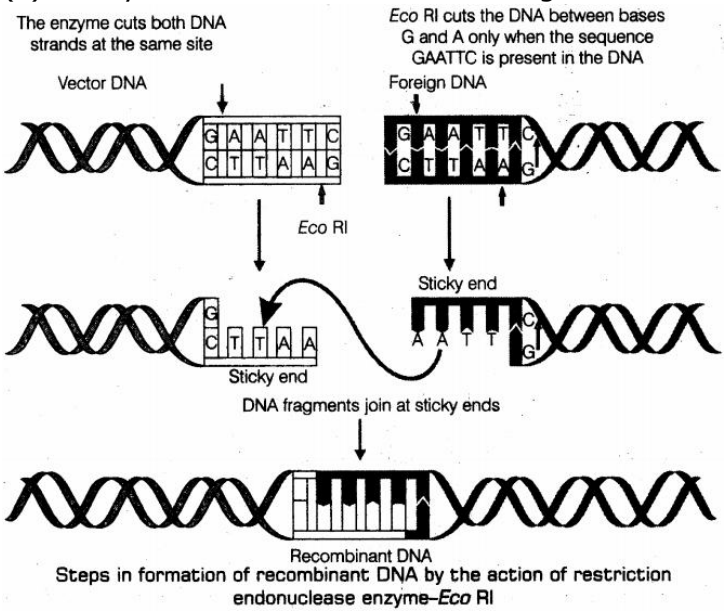
(ii) Recognition sequences These are specific base sequences of DNA, where restriction enzyme cuts the DNA. They are utilised to extract the desired gene or fragments from DNA molecules.

(iii) Gel electrophoresis It is a technique, used to separate the DNA fragments according to their size through seiving effect of the gel.**47. EcoRI 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.**

- **The set of palindromic nucleotide sequence of base pairs the EcoRI will recognise in both the DNA segments. Mark the site at which EcoRI will act and cut both the segments.**
- **Sticky ends formed on both the segments, where the two DNA segments will join later to form a recombinant DNA. [Delhi 2010]**

Ans. Palindromic sequence of Eco Rl is

5′ G AATTC 3′
3′C TTAA G 5′ t
(the arrows indicate the site, where it cuts the strands.)
(ii) Sticky ends formed on both the segments.

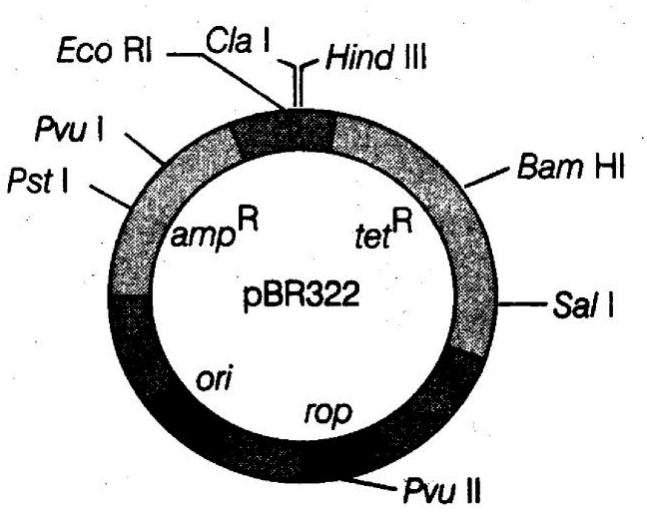


Steps in formation of recombinant DNA by the action of restriction endonuclease enzyme–*Eco* RI

(a) Restriction enzymes cut the strand of DNA a little away from the centre of the palindrome sites, but between the same two bases on the opposite strands.
(b) This leaves single stranded portions at the ends.
(c) There are overhanging stretches called sticky ends on each strands as given in above figure. These are named so, because they form hydrogen bonds with their complementary cut counterparts.
(d) The stickiness of the ends facilitates the action of the enzyme DNA ligase.
(e) Restriction endonucleases are used in genetic engineering to form recombinant molecules of DNA, which are composed of DNA from different sources/genomes.
(f) These sticky ends are complementary to each other when cut by same restriction enzyme,

therefore can be joined together (end-to-end) using DNA ligases.**48. (i) Name the organism in which the vector shown is inserted to get the copies of the desired gene.**

- **Mention the area labelled in the vector responsible for controlling the copy number of the inserted gene.**
- **Name and explain the role of aselectable marker in the vector Shown. [All India 2010]**



Ans. (i) Escherichia coli (E. coli)
(ii) Ori in the vector is responsible for controlling the copy number of inserted gene.
(iii) The genes encoding resistance to antibiotics like tet^R resistant to tetracycline, amp^R resistant to ampicillin are used as selectable markers. If a foreign DNA is ligated at the Bam HI site of tetracycline resistance gene, the recombinant plasmids will lose the tetracycline resistance.
The selectable markers help in identifying and eliminating non-transformants and selectively permitting the growth of transformants.

49. (i) EcoRI is a restriction endonuclease. How is it named so? Explain.
(ii) Write the sequence of DNA bases that the enzyme recognises. Mention the point at which the enzyme makes a cut in the DNA segment. [Delhi 2010c]

Ans.(I) Naming of restriction endonuclease are:
(i) The first letter of the name comes from the genus and next two letters from species of the prokaryotic cell from where enzymes are extracted.
(ii) The Roman numbers following the name show the order in which the enzymes, were isolated from the bacterial strain. For example, Eco Rl is derived from Escherichia coli RY 13, Hind II from Haemophilus influenzae Rd, etc.
(II) The recognition sequence is a palindrome, where the sequence of the base pair reads the same on both the DNA strands, when orientation of reading is kept same,
e.g., 5′- GAATTC -3′
3′- CTTAAG -5′
The enzyme Eco Rl it cuts the DNA segment at bases G and A on both the strands only when the sequence GAATTC

50. (i) Name the technique used for separation of DNA fragments.

- **Write the type of matrix used in this technique.**

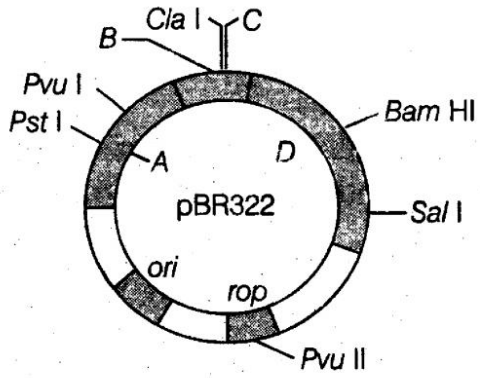
- **How is the separated DNA visualised and extracted for use in recombinant technology? [All India 2010]**

Ans. (i) Gel electrophoresis.

(ii)The material used as matrix in gel electrophoresis is agarose.
This agarose gel acts as a sieve to separate the DNA fragments according to their size.

(iii) The separated DNA fragments are stained with ethidium bromide.
(a) By the exposure to UV radiation, the separated DNA fragments become visible as orange-coloured bands.
(b) The separated bands of DNA are cut out from the agarose gel and DNA is extracted from these gel pieces, this process is called elution.

51. (i) Identify the selectable markets in the diagram of E. coli vector shown below.



(*ii*) How is the coding sequence of α-galactosidase considered a better marker than the ones identified by you in the above diagram. Explain. **[Delhi 2009]**

Ans. (i) A – Ampicillin resistanceD – Tetracycline resistance are used as selectable markers in E.coli cloning vector.
(ii) Coding sequence of a-galactosidase is a better marker, as the recombinants and non-recombinants are differentiated on the basis of their ability to produce colour in the presence of a chromogenic substrate, while the selection of recombinants due to inactivation of antibiotic resistant gene is a tedious and time taking process to grow them simultaneously on two antibiotics separately.
(a) Introduction of rDNA into the coding sequence of a-galactosidase leads to insertional inactivation.
(ii) The recombinants do not produce a blue colour, while the non-recomninants produces a blue colour.

52. Name and explain the techniques used in the separation and isolation of DNA fragments to be used in recombinant DNA technology.
[All India 2009]

Ans.DNA fragments formed by the use of restriction endonucleases are separated by gel electrophoresis.
(i) DNA fragments are negatively charged molecules. Thus, they move towards the anode under electric field through the medium.

(ii) DNA fragments separate according to their size due to seiving effect of agarose gel.
(iii) The separated DNA fragments can be viewed by staining the DNA with ethidium bromide followed by exposure to UV radiation.
(iv) The separated bands of DNA are cut and extracted from gel piece. This is known as elution.

53. Why are genes encoding resistance of antibiotics considered useful selectable markers for coli cloning vector? Explain with the help of one example. [Delhi 2009c]

Ans. The genes encoding resistance to antibiotics are cosidered useful selectable markers because the normal £. coli cells do not carry resistance against any of these antibiotics.

Example A foreign DNA is ligated at the Bam HI site of tetracycline resistance gene in the vector pBR322. The recombinant plasmids will lose tetracycline resistance due to the insertion of foreign DNA but can still be selected from non-recombinants by plating the transformants on ampicillin containing medium.
The transformants growing on ampicillin containing medium are then transferred on medium containing tetracycline. The recombinants will not grow, while non-recombinants will grow on the medium containing both the antibiotics. Antibiotic resistance gene, thus helps in selecting the transformants.

54. (i) Write the palindromic nucleotidesequence for the following DNA segment
5'- GAATTC- 3'

- **Name the restriction endonuclease that recognises this sequence.**
- **How are sticky-ends produced? Mention their role. [All India 2009]**

Ans. (i) Palindromic sequence for

5'- GAATTC- 3'
3'- CTTAAG -5'.
(ii) Restriction endonuclease Eco Rl recognises the above palindromic sequence.
(iii)Sticky ends on DNA are formed by action of enzymes restriction endonucleases. These enzymes cut the strand of DNA a little away from the centre of the palindrome sequence between the same two bases on both the strands. This results in single stranded stretches on both the complementary strands at their ends.
These overhanging stretches are called sticky ends as they form hydrogen bonds with the complementary base pair sequences.

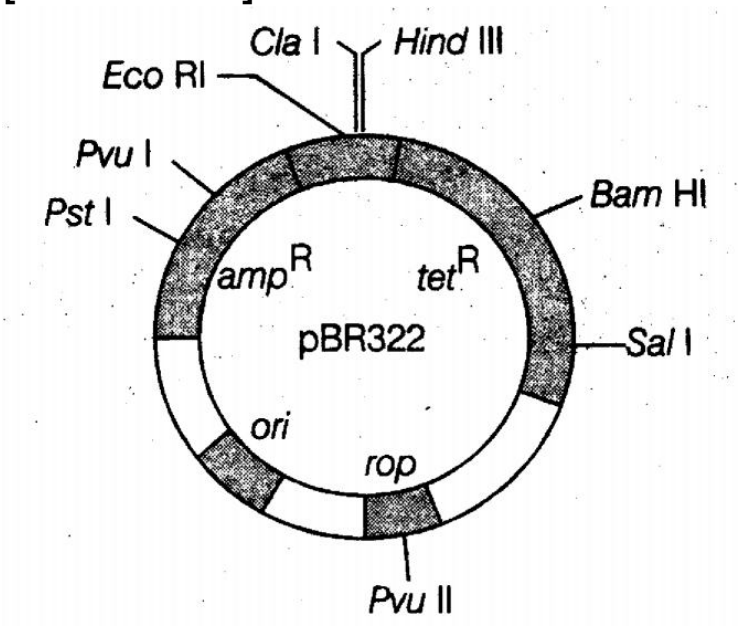
Role of the sticky ends These sticky ends produced form hydrogen bonds with their complementary cut counterparts. This stickiness of the ends faciIitates the action of the enzyme DNA ligase.

55. (i) What are molecular scissors?
Give one example.
(ii) Explain their role in recombinant DNA technology.
[Delhi 2008; Foreign 2008]
or
(i) Name the category of enzymes that cut at a specific site within the DNA molecule. Give an example.
(ii) Explain, how do these enzymes function? Mention their use in genetic engineering. [All India 2008C]

Ans. (i) Molecular scissors are the restriction endonucleases because they cut the DNA segments at particular locations or specific sequence, e.g. Eco Rl.

(ii) The restriction enzymes cut the DNA strand 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 at the ends with overhanging stretches called sticky ends on each strand. These ends form hydrogen bonds with their complementary cut counterparts. This stickiness of the ends facilitates the action of the enzyme DNA ligase.

56. Why is Agrobacterium tumefaciens a good cloning vector? Explain.
[All India 2008]



Ans. Agrobacterium tumefaciens is a soil bacterium, which causes disease in many dicot plants. It is a natural vector as 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 cellsto produce the chemicals required by the pathogen.
The Tumour inducing (Ti) plasmid of A. tumefaciens has now been modified into a cloning vector, which is no more pathogenic to the plants but still deliver the gene of interest, that gets incorporated into genome of plant.

57. Explain the importance of
(i) ori (ii) amp^R and (iii) rop in the E.coli vector shown below.
[All India 2008]

Ans.(i) Ori is the sequence of DNA 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 linked DNA.
(ii) amp^R is an antibiotic resistance gene in which ligation of alien DNA is carried out at a restriction site.
(iii) rop codes for the proteins involved in the replication of the plasmid.

58. A vector is engineered with three features, which facilitate its clonincj within the host cell. List the three features and explain each of them.
[Foreign 2008]

Ans. Features which facilitate cloning of vector are:
(i) **Origin of replication** (Ori)

- This is the sequence of DNA from where replication starts.
- Any piece of alien/foreign DNA linked to it is made to replicate within host cell. It also decides the copy number of the linked[DNA.
  (ii) **Selectable marker** is a marker gene, which helps in selecting the host cells, which are transformants/recombinants from the non-recombinant ones.
  For example, ampicillin and tetracycline resistant genes in, £. coli.
  (iii) Cloning site is a unique recognition site in a vector to link the foreign DNA. The presence of a particular cloning/recognition site helps the partjcular restriction enzyme to cut the vector DNA. Single recognition site is commonly preferred.
  (iv) **Small size of the vector** The small size facilitates the introduction of the DNA into the host easily.

59. Why a cell must be made competent to take up DNA?Explain the steps by which a bacterial cells made competent to take up plasmid/rDNA. [Delhi 2008C]

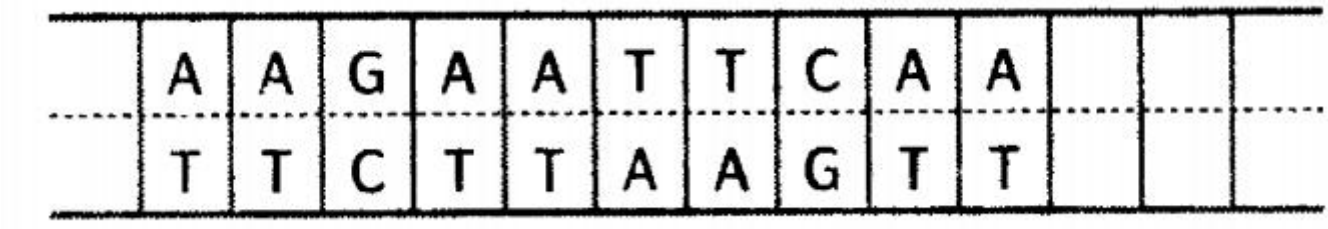
Ans. DNA being a hydrophilic molecule cannot pass through cell membrane. Hence, the bacterial cell is made competent to accept the DNA molecule. Thus, competency is the ability of a cell to take up foreign DNA.
Following methods are used to make a bacterial cell competent:
the bacteria should be made competent to accept the DNA molecules,

(i) Competency is the ability of a cell to take up foreign DNA.
(ii) Methods to make a cell competent are as follow.

(a) Chemical method In this method, the cell is treated with a 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. This is called heat shock treatment.
  • This enables the bacteria to take up the recombinant DNA.
  (b) Physical methods In this method, a recombinant DNA is directly injected into the nucleus of an animal cell by microinjection method.
  • In plants, cells are bombarded with high velocity microparticles of gold or tungsten coated with DNA called as biolistics or gene gun method.
  (c) Disarmed pathogen vectors when allowed to infect the cell, transfer the recombinant DNA into the host.

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



- **What is called a palindromic sequence in a DNA?**
- **Write the palindromic nucleotide sequence shown in the DNA strand given and mention the enzyme that will recognise such a sequence.**

- **State the significance of enzymes that identify palindromic nucleotide sequences. [All India 2008C]**

Ans. (i) Palindromic sequence in a DNA are base sequences that reads same, both in forward and backward direction.
(ii) Palindromic sequence in DNA 5′- GAATTC- 3′
3 – CTTAAG- 5′
The sequence reads the same on the two strands in 5′-> 3′ direction. This is also same if read in the 3′->5′ direction. Restriction endonuclease recognises such sequence.
DNA being a hydrophilic molecule cannot pass through cell membrane. Hence, the bacterial cell is made competent to accept the DNA molecule. Thus, competency is the ability of a cell to take up foreign DNA.
Following methods are used to make a bacterial cell competent:
the bacteria should be made competent to accept the DNA molecules,

(i) Competency is the ability of a cell to take up foreign DNA.
(ii) Methods to make a cell competent are as follow.

(a) Chemical method In this method, the cell is treated with a 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. This is called heat shock treatment.
• This enables the bacteria to take up the recombinant DNA.
(b) Physical methods In this method, a recombinant DNA is directly injected into the nucleus of an animal cell by microinjection method.
• In plants, cells are bombarded with high velocity microparticles of gold or
tungsten coated with DNA called as biolistics or gene gun method.
(c) Disarmed pathogen vectors when allowed to infect the cell, transfer the recombinant DNA into the host.
Significance of restriction endonucleases They are used in genetic engineering to form recombinant molecules of DNA,
which are composed of DNA from different sources/genomes and allow isolating the desired gene fragment and joining it with host on vector DNA due to sticky ends generated.

5 Marks Questions

61. (i) Describe the characteristics 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? [All India 2011]
Ans.(I) The following features are required tofacilitate cloning into a vector
Features which facilitate cloning of vector are:
(i) **Origin of replication** (Ori)

- This is the sequence of DNA from where replication starts.
- Any piece of alien/foreign DNA linked to it is made to replicate within host cell. It also decides the copy number of the linked[DNA.

(ii) **Selectable marker** is a marker gene, which helps in selecting the host cells, which are transformants/recombinants from the non-recombinant ones.
For example, ampicillin and tetracycline resistant genes in, £. coli.
(iii) Cloning site is a unique recognition site in a vector to link the foreign DNA. The presence of a

particular cloning/recognition site helps the partjcular restriction enzyme to cut the vector DNA. Single recognition site is commonly preferred.

(iv) **Small size of the vector** The small size facilitates the introduction of the DNA into the host easily.

(II) DNA being a hydrophilic molecule cannot pass through cell membrane. Hence, the bacterial cell is made competent to accept the DNA molecule. Thus, competency is the ability of a cell to take up foreign DNA.
Following methods are used to make a bacterial cell competent:
the bacteria should be made competent to accept the DNA molecules,

(i) Competency is the ability of a cell to take up foreign DNA.
(ii) Methods to make a cell competent are as follow.

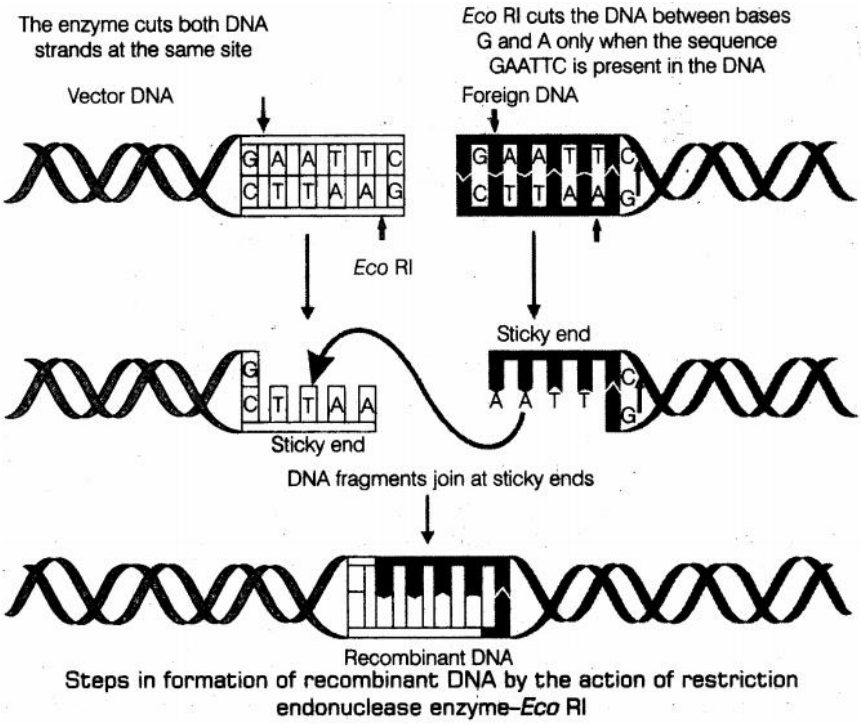
(a) Chemical method In this method, the cell is treated with a specific concentration of a divalent cation such as calcium to increase pore size in cell wall.

- 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. This is called heat shock treatment.
• This enables the bacteria to take up the recombinant DNA.
(b) Physical methods In this method, a recombinant DNA is directly injected into the nucleus of an animal cell by microinjection method.
• In plants, cells are bombarded with high velocity microparticles of gold or tungsten coated with DNA called as biolistics or gene gun method.
(c) Disarmed pathogen vectors when allowed to infect the cell, transfer the recombinant DNA into the host.

62. (i) With the help of diagrams show the different steps in the formation of recombinant DNA by action of restriction endonuclease enzyme Eco RI.
(ii) Name the technique that is used for separating the fragments of DNA cut by restriction endonucleases. [All India 2011]

Ans. (i) Formation of recombinant DNA by the action of restriction endonuclease enzyme Eco Rl. The restriction enzyme cuts both DNA strands at the same site, producing sticky ends.



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

(a) Restriction enzymes cut the strand of DNA a little away from the centre of the palindrome sites, but between the same two bases on the opposite strands.
(b) This leaves single stranded portions at the ends.
(c) There are overhanging stretches called sticky ends on each strands as given in above figure. These are named so, because they form hydrogen bonds with their complementary cut counterparts.
(d) The stickiness of the ends facilitates the action of the enzyme DNA ligase.
(e) Restriction endonucleases are used in genetic engineering to form recombinant molecules of DNA, which are composed of DNA from different sources/genomes.
(f) These sticky ends are complementary to each other when cut by same restriction enzyme, therefore can be joined together (end-to-end) using DNA ligases.
(ii) Gel electrophoresis.

63. (i) Why are engineered vectors preferred by biotechnologists for transferring the desired genes into another organism?(ii) Explain, how do ori, selectable marker and cloning sites facilitate cloning into a vector? [Foreign 2009]
Ans. (I) Engineered vectors are preferred by biotechnologists because they help in easy linking of foreign DNA and selection of recombinants from non-recombinants.
(II) Features which facilitate cloning of vector are:

(i) **Origin of replication** (Ori)

- This is the sequence of DNA from where replication starts.
- Any piece of alien/foreign DNA linked to it is made to replicate within host cell. It also decides the copy number of the linked[DNA.

(ii) **Selectable marker** is a marker gene, which helps in selecting the host cells, which are transformants/recombinants from the non-recombinant ones.
For example, ampicillin and tetracycline resistant genes in, £. coli.

(iii) Cloning site is a unique recognition site in a vector to link the foreign DNA. The presence of a particular cloning/recognition site helps the partjcular restriction enzyme to cut the vector DNA. Single recognition site is commonly preferred.

(iv) **Small size of the vector** The small size facilitates the introduction of the DNA into the host easily.

Processes of Recombinant DNA Technology

1 Mark Questions

1.Name the enzymes that are used for the isolation of DNA from bacterial and fungal cells for recombinant DNA technology.
[All India 2014; Foreign 2014]

Ans. The enzymes used for the isolation of DNA from bacterial cells is lysozyme and fungal cells is chitinase.

2.How can bacterial DNA be released from the bacterial cell for biotechnology experiments? [Delhi 2011]

Ans. Bacterial cells are treated with lysozyme to digest the cell wall for releasing DNA.

3. Why is the enzyme cellulase used for isolating genetic material from plant cells but not for animal cells? [Delhi 2010]

Ans. Cellulase is used for digesting the cellulosic cell wall of plant cells. Animal cells do not contain cell wall, so cellulase is not required.

4. What is the host called that produce a foreign gene product? What is this product called? [Foreign 2010]

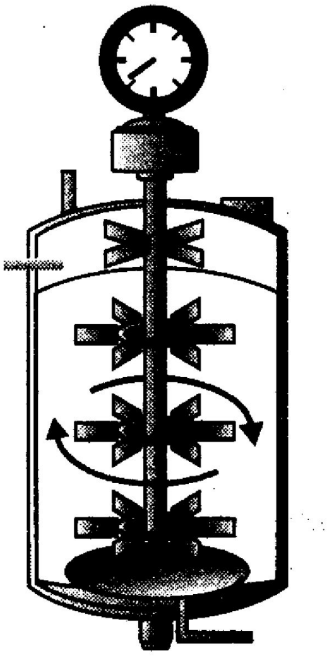
Ans. The host cells that produce foreign gene product are called transgenic organisms or Genetically Modified Organisms (GMOs). The product is called recombinant proteins.

2 Marks Questions

5. Name the source of the DNA polymerase used in PCR technique. Mention why it is used? [All India 2013,2012; Foreign 2011]

Ans. The DNA polymerase used in PCR is Tag polymerase extracted from Thermus aquaticus. It is a thermostable enzyme that can withstand high temperature used in the denaturation and separation of DNA strands. Hence, it can be used for a number of cycles in amplification.

6. Name the type of bioreactor shown. Write the purpose for which it is used? [All India 2011]



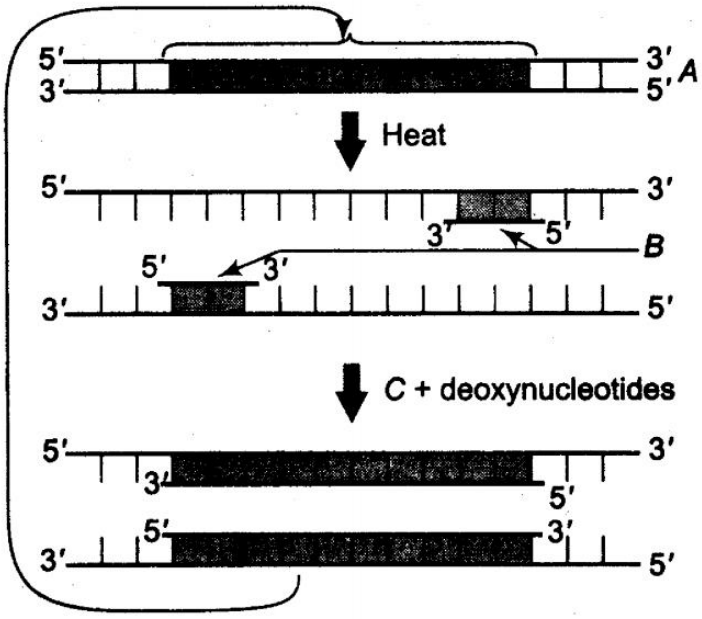
Ans. Figure is a simple stirred-tank bioreactor.
Bioreactors are used to produce large quantities of the desired gene products

7. (i) Mention the number of primers required in each cycle of Polymerase Chain Reaction (PCR). Write the role of primers and DNA polymerase in PCR.
(ii) Give the characteristic feature and source organism of the DNA polymerase used in PCR. [All India 2010]

Ans. (i) Two sets of primers are required. Primers are required for the addition of nucleotides to make multiple copies of the DNA of interest. The enzyme DNA polymerase extends the primers using the nucleotide provided.

(ii) The DNA polymerase is thermostable and withstands the high temperature treatment during denaturation of the DNA
it is obtained from a bacterium, Thermus aquaticus.

8. A schematic representation of Polymerase Chain Reaction (PCR) up to the extension stage is given below. Answer the questions that follow



(i) Name the process
(ii) Identify B.
(iii) Identify C and mention its importance in PCR. [Foreign 2010]

Ans. (i) A-Denaturation of the double stranded DNA.

(ii) B-Primers

(iii) C-DNA polymerase or Taq polymerase. **Importance in PCR**
It extends the primers using the nucleotides provided in the reaction medium and the genomic DNA as the template. Taq polymerase is thermostable and withstands the high temperature used in denaturation process.

9. Any recombinant DNA with a desired gene is required in billion copoies for commercial use. How is the amplification done? Explain. [Delhi 2010c]

Ans. Amplification of gene is done using polymerase Chain Reaction (PCR). it is carried out in the following steps:

(i) Denaturation The double stranded DNA is denatured by applying high temperature of 95°C for 15 seconds. Each separated strand acts as a template.

(ii) Annealing Two sets of primers are added, which anneal to the 3′ end of each separated strand.

(iii) Extension DNA polymerase extends the primers by adding nucleotides complementary to the template provided in the reaction. Taq polymerase is used in the reaction, which can tolerate heat. All these steps are repeated many times to get several copies of the desired DNA.

10. Explain the contribution of Thermus aquaticus in the amplification of a gene of interest. [Delhi 2009]

Ans. Thermus aquaticus provides thermostable DNA polymerase. It can withstand the high temperature used in denaturation and separation of DNA strands during Polymerase Chain Reaction (PCR). Hence, can be used for repeated amplification of DNA.

11. What are recombinant proteins? How do bioreactors help in their production? [All India 2009]

Ans. Recombinant proteins are produced by the expression of recombinant DNA in the transgenic organism. Bioreactors help in producing these proteins on a large scale as
(i) Large volumes of culture can be processed in bioreactors to produce desired quantity of product.
(ii) These provide optimum conditions of pH, oxygen, salts, substrates, etc., to get the desired product.

12. Mention the three steps involved in each cycle of Polymerase Chain Reaction (PCR). How is repeated amplification of DNA made possible using PCR? [All India 2008C]
Ans. (i) A-Denaturation of the double stranded DNA.
(ii) B-Primers
(iii) C-DNA polymerase or Taq polymerase.

Importance in PCR
It extends the primers using the nucleotides provided in the reaction medium and the genomic DNA as the template. Taq polymerase is thermostable and withstands the high temperature used in denaturation process.
Repeated amplification of DNA in PCR is made possible by using thermostable DNA polymerase, which remain active during high temperature.

3 Marks Questions

13. (i) List the three steps involved in Polymerase Chain Reaction (PCR).
(ii) Name the source organism of Taq polymerase.
Explain the specific role of this enzyme in PCR. [Foreign 2014]

Ans. (I)Amplification of gene is done using polymerase Chain Reaction (PCR). it is carried out in the following steps:

(i) Denaturation The double stranded DNA is denatured by applying high temperature of 95°C for 15 seconds. Each separated strand acts as a template.

(ii) Annealing Two sets of primers are added, which anneal to the 3′ end of each separated strand.

(iii) Extension DNA polymerase extends the primers by adding nucleotides complementary to the template provided in the reaction. Taq polymerase is used in the reaction, which can tolerate heat. All these steps are repeated many times to get several copies of the desired DNA.

(II) (i) The DNA polymerase is thermostable and withstands the high temperature treatment during denaturation of the DNA
C-DNA polymerase or Taq polymerase. **Importance in PCR**
It extends the primers using the nucleotides provided in the reaction medium and the genomic DNA as the template. Taq polymerase is thermostable and withstands the high temperature used in denaturation process.

14. (i) What is a bioreactor? How does it work? (ii) Name two commonly used bioreactors. [Delhi 2014c]

Ans. (i) Bioreactors are large vessels in which raw materials are biologically converted into specific products by microbes, plant and animal cells or human cells.
The bioreactors work by providing optimal conditions to process the culture as well as the production of desired product by maintaining optimum pH, temperature, oxygen and other growth conditions required.

(ii) The two commonly used bioreactors are:
(a) Simple stirred-tank bioreactors
(ii) Sparged stirred-tank bioreactors.

15. What is a bioreactor used for? Name a- commonly used bioreactor and any two of its components? [All India 2014C]

Ans. A bioreactor is used for converting the raw materials into specific products biologically such as proteins, enzymes, etc., through the use of microbial, plant or animals cells.
The most commonly used bioreactors are of stirred type.
The two components of a stirred tank bioreactor are:
(i) In let for sterile air or oxygen
(ii) Agitator system
(iii) Temperature control system
(iv) pH control system
(v) Foam control system
(vii) Sampling ports (choose any two)

16. How is the amplification of a gene sample of interest carried out using Polymerase Chain Reaction (PCR)? [All India 2012]
or
Describe the process of gene amplification for rDNA technology experiments. [All India 201 ic]

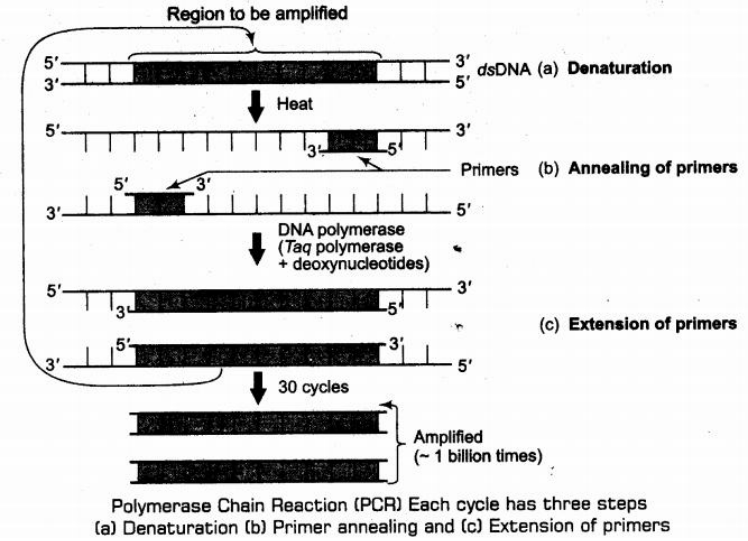
Ans. Amplification of gene is done using polymerase Chain Reaction (PCR). it is carried out in the following steps:

(i) Denaturation The double stranded DNA is denatured by applying high temperature of 95°C for 15 seconds. Each separated strand acts as a template.

(ii) Annealing Two sets of primers are added, which anneal to the 3′ end of each separated strand.

(iii) Extension DNA polymerase extends the primers by adding nucleotides complementary to the template provided in the reaction. Taq polymerase is used in the reaction, which can tolerate

heat. All these steps are repeated many times to get several copies of the desired DNA.



Polymerase Chain Reaction (PCR) Each cycle has three steps
(a) Denaturation (b) Primer annealing and (c) Extension of primers

17. How is the bacterium Thermus aquaticus employed in recombinant DNA technology? [All India 2008]

Ans. Thermus aquaticus, a bacterium yields DNA polymerase used in PCR in recombinant DNA technology.
(i) This enzyme remains active during the high temperature applied in the denaturation of double stranded DNA.
(ii) It extends the primers using the nucleotides provided in the reaction and the genomic DNA as template.
(iii) Repeated amplification is achieved by this enzyme. The amplified fragments, if desired can be used to ligate with a vector for further cloning.

18. What are bioreactors? List five growth conditions that a bioreactor provides for obtaining the desired product. [Delhi 2008C]

Ans. (i) Bioreactors are large vessels in which raw materials are biologically converted into specific products by microbes, plant and animal cells or human cells.
The bioreactors work by providing optimal conditions to process the culture as well as the production of desired product by maintaining optimum pH, temperature, oxygen and other growth conditions required.
Growth conditions that a bioreactor provides for obtaining desired product are:
(i) Optimum temperature
(ii) Suitable pH
(iii) Salt
(iv) Vitamins
(v) Oxygen

5 Marks Questions

19. 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. [All India 2011]

Ans. (i) Cutting of desired gene at specific location is done by incubating the DNA with specific restriction endonuclease. Restriction enzymes recognise a particular palindromic nucleotide sequence and cuts the DNA at that site.
(ii) Synthesis of multiple copies of desired gene is carried out by Polymerase Chain Reaction (PCR).
Amplification of gene is done using polymerase Chain Reaction (PCR). it is carried out in the following steps:

(i) Denaturation The double stranded DNA is denatured by applying high temperature of 95°C for 15 seconds. Each separated strand acts as a template.

(ii) Annealing Two sets of primers are added, which anneal to the 3' end of each separated strand.

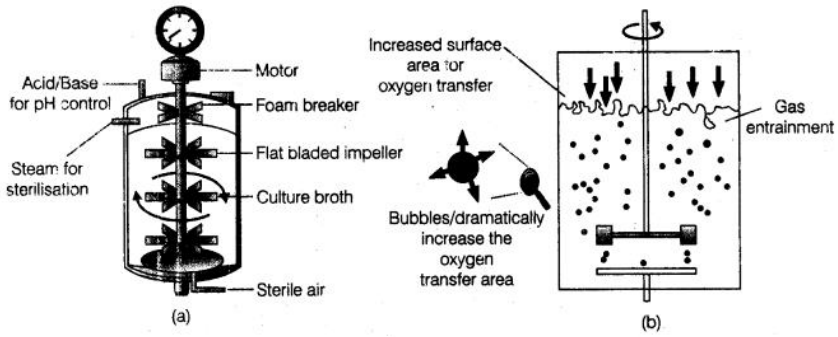
(iii) Extension DNA polymerase extends the primers by adding nucleotides complementary to the template provided in the reaction. Taq polymerase is used in the reaction, which can tolerate heat. All these steps are repeated many times to get several copies of the desired DNA.

20. Name the source of taq polymerase.
Explain the advantage of its use in biotechnology. [All India 2009]

Ans. Taq polymerase is Pbtained from the bacterium Thermus aquaticus.
The enzyme is thermostable and can withstand the high temperature used for denaturation and separation of the two strands of DNA in PCR. Desired gene can be amplified to produce even a billion copy of DNA.
Thermus aquaticus, a bacterium yields DNA polymerase used in PCR in recombinant DNA technology.
(i) This enzyme remains active during the high temperature applied in the denaturation of double stranded DNA.
(ii) It extends the primers using the nucleotides provided in the reaction and the genomic DNA as template.
(iii) Repeated amplification is achieved by this enzyme. The amplified fragments, if desired can be used to ligate with a vector for further cloning.

21. What is a bioreactor? Draw a labelled diagram of a sparged stirred bioreactor. Explain its functioning. [All India 2009c]

Ans. (i) Bioreactors are large vessels in which raw materials are biologically converted into specific products by microbes, plant and animal cells or human cells.
The bioreactors work by providing optimal conditions to process the culture as well as the production of desired product by maintaining optimum pH, temperature, oxygen and other growth conditions required.
and



(a Simple stirred-tank bioreactor (b) Sparged stirred-tank bioreactor through, which sterile air bubbles are sparged

In the sparged stirred-tank bioreactor, sterile air bubbles are sparged. This increases the surface * sea for oxygen transfer, thus facilitating the growth and metabolism of cells and hence, productions of recombinant products as well.

Miscellaneous Questions

3 Marks Questions

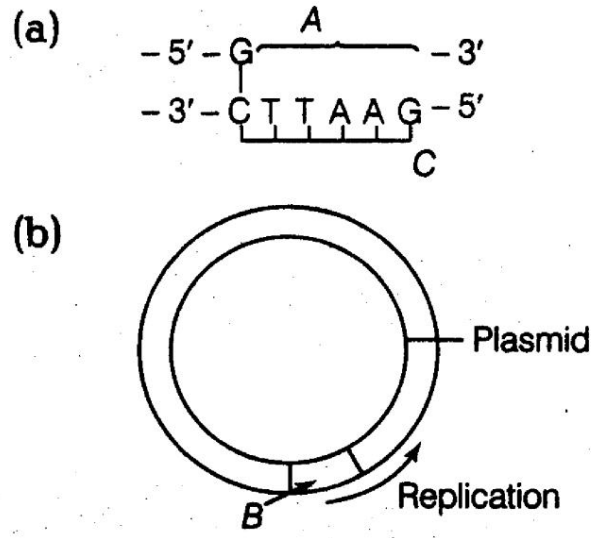
1.Explain the basis on which the gel electrophoresis technique works. Write any two ways the products obtained through this technique can be utilised. [Delhi 2013C]

Ans. Gel electrophoresis technique works on the principle of separation of DNA fragment on the basis of electric charge.
Since, DNA is negatively charged molecule so, they can be forced to separate out according to their size towards anode under an electric field through a medium or matrix (commonly used is agarose). Shorter molecule moves faster and migrate further than the lohger one.
The products obtained through this technique can be utilised as follows:
(i) Construction of recombinant DNA by joining with cloning vectors.
(ii) Used in making multiple copies of same DNA by using PCR (Polymerase Chain Reaction).

2. How can the following be made possible for biotechnology experiments?
(i) Isolation of DNA from bacterial cell.
(ii) Reintroduction of the recombinant DNA into a bacterial cell.
[Foreign 2012]

Ans. (i) Isolation of DNA from bacterial cell can be done by:
(a) treating the bacterial cells with enzymes such as lysozyme to remove cell wall.
(b) the RNA associated with DNA can be removed by treatment with ribonuclease, whereas protein can be removed by treatment with protease. Similarly other molecules (if any) are removed by appropriate treatment.
(ii) Reintroduction of the recombinant DNA into bacterial cell can be done by the following methods:
(a) The recipient bacterial cell is made 'competent' to take up the recombinant DNA by treatment with a specific increase in concentration of calcium ions.
(b) the recombinant DNA is forced into the cells by heat shock treatment, i.e. by incubating the cells with rDNA followed by placing them at 42°C (heat shock) and then putting them back on ice. This enables bacteria to take up rDNA.

3. (i) Identify A and B illustrations in the following:



(ii) Write the term given to A and C and why?
(iii) Expand PCR. Mention its importance in biotechnology. [Delhi 2011]

Ans. (i) (a) A is recognition or restriction site (AATTC), which is recognised by restriction enzyme Eco
(b) B is rop gene protein involved in the replication of plasmid coded by this gene.

(ii) A and C are called palindromes. These are sequence of base pairs that reads same on the two strands of DNA, when orientation of reading is kept same.

(iii) PCR is polymerase chain reaction, multiple copies of the gene of interest can be synthesised in vitro by this technique. Thus, PCR can be utilised to amplify a single gene or fragment into thousands of copies to be used in cloning experiments.