UNIT-IX

Biotechnology and its Applications

CHAPTER 11

BIOTECHNOLOGY: PRINCIPLES AND PROCESSES

Syllabus

➢ ***Principles and processes of biotechnology :*** *Tools of Recombinant DNA technology, Process of r-DNA technology*

Chapter Analysis

List of Topics		2016		2017		2018
		D	OD	D	OD	D/OD
Tools of rDNA technology	• Role of restriction enzymes, palindromic sequences, plasmids, DNA ligase, Gel electrophoresis • Steps of rDNA technology to obtain a foreign product • Selectable markers in pBR 322 • Competent host • Methods to introduce alien DNA into host cells	1 Q (1 M)/ 1 Q (2 M)	2 Q (3 M)	2 Q (3 M)	1 Q (3 M)	1 Q (3 M)
Processes of rDNA technology	• Polymerase chain reaction • Bioreactor				1 Q (3 M)	1 Q (3 M)

- *On the basis of above analysis, it can be concluded that mostly a three mark question is usually asked from this chapter. The important topics are- tools of rDNA technology (restriction enzymes, plasmids, DNA ligase, gel electrophoresis, selectable markers in pBR322, identification of recombinants, palindromic sequences), steps of PCR, methods to introduce alien DNA into competent host and bioreactor. Don't forget to practice the diagram showing steps of rDNA technology.*

TOPIC-1
Principles of Biotechnology and Tools of Recombinant DNA Technology

Revision Notes

Introduction

- **Biotechnology** deals with techniques of using live organisms or their enzymes for products and processes useful to humans.
- The term biotechnology was given by Karl Ereky (1919).
- The **European Federation of Biotechnology (EFB)** defines Biotechnology as 'the integration of natural science and organisms, cells, parts there of, and molecular analogues for products and services'.
- **Biotechnology deals with :**
  - Microbe-mediated processes (making curd, bread, wine etc).

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Process of Recombinant DNA Technology .... P. 292

- *In vitro* fertilisation ('test-tube' baby programme).
- Synthesis and using of a gene.
- Preparation of a DNA vaccine.
- Correcting a defective gene.

Principles of Biotechnology

- **The two core techniques of modern biotechnology are :**
  - **(a) Genetic engineering :** The technique in which the genetic material (DNA and RNA) is chemically altered and introduced into host organisms to change the phenotype is known as genetic engineering.
  - **(b) Maintenance of sterile ambience :** It is necessary in chemical engineering processes for growing only the desired microbe / eukaryotic cell in large quantities for the manufacture of antibiotics, vaccines, enzymes, etc.
- Traditional hybridisation techniques lead to inclusion and multiplication of undesirable genes along with desired genes.
- Genetic engineering helps to isolate and introduce only desirable genes into the target organism.
- A piece of DNA is not only able to multiply itself in the progeny cells of the organism. But, when it gets integrated into the recipient genome, it multiplies and inherits along with the host DNA.
- First recombinant DNA was emerged from the possibility of linking a gene of antibiotic resistance with a native plasmid of *Salmonella typhimurium*. Plasmid is an autonomously replicating circular extra chromosomal DNA.
- Stanley Cohen and Herbert Boyer (1972) constructed first recombinant DNA. They isolated the antibiotic resistance gene by cutting out a piece of DNA from a plasmid.

Steps in Genetically Modifying an Organism

- **There are three basic steps in genetically modifying an organism :**
  - **(a)** Identification of DNA with desirable genes.
  - **(b)** Introduction of the identified DNA into the host.
  - **(c)** Maintenance of introduced DNA in the host and transfer of the DNA to its progeny.

Tools of Recombinant DNA technology

1. Restriction Enzymes ('molecular scissors')

- The restriction enzymes are called molecular scissors and are responsible for cutting DNA.
- In 1963, two enzymes responsible for restricting the growth of bacteriophage in *E. coli* were isolated. One of these added methyl groups to DNA. The other (restriction endonuclease) cut DNA.
- The first restriction endonuclease is Hind II. Isolated by Smith, Wileox and Kelley (1968) from *Haemophilus influenzae* bacterium. It always cuts DNA molecules at a particular point by recognizing a specific sequence of six base pairs. This is known as the recognition sequence for Hind II.
- Today more than 900 restriction enzymes have been isolated from over 230 strains of bacteria.

Naming of the restriction enzymes

- First letter indicates genus and the second two letters indicate species of the prokaryotic cell from which they were isolated *e.g. EcoRI* comes from *E. coli* RY 13, where R = the strain, Roman numbers = the order in which the enzymes were isolated from that strain of bacteria.
- Restriction enzymes belong to a class of enzymes called **nucleases**.
- The nucleases include **exonucleases** and **endonucleases**.

(i) Exonucleases

They remove nucleotides from the ends of the DNA.

(ii) Endonucleases

- They cut at specific positions within the DNA.
- Each restriction endonuclease can bind to specific recognition sequence of the DNA and cut each of the two strands at specific points in their sugar-phosphate backbones.
- Each restriction endonuclease recognizes a specific palindromic nucleotide sequences in the DNA.
- The palindrome in DNA is a sequence of base pairs that read the same on the two strands in 5′ → 3′ direction and in 3′ → 5′ direction. *e.g.*

  5′ —— GAATTC —— 3′

  3′ —— CTTAAG —— 5′
- Restriction enzymes cut the strand a little away from the centre of the palindrome sites but between the same two bases on the opposite strands. This leaves single stranded overhanging stretches at the ends. They are called sticky ends.
- They form H-bonds with their complementary cut counterparts. This stickiness facilitates action of the enzyme **DNA ligase**.

- When cut by the same restriction enzyme, the resultant DNA fragments have the same kind of sticky-ends and these are joined together by the enzyme DNA ligases.

Separation and isolation of DNA fragments :

- DNA fragments formed by restriction endonucleases can be separated by a technique called **gel electrophoresis**.
- DNA fragments are negatively charged. So, they can be separated by moving them towards the anode under an electric field through a medium / matrix such as **agarose** (which is a natural polymer of D-galactose and 3, 6 anhydro L-galactose and which is extracted from sea weeds).
- The DNA fragments separate (resolve) according to their size through sieving effect provided by the agarose gel.
- The smaller sized fragments move farther.
- The separated DNA fragments can be visualized after staining the DNA with **ethidium bromide** followed by exposure to UV radiation. Bright orange coloured DNA bands can be seen.
- The separated DNA bands are cut out from agarose gel and extracted from gel piece. This step is called **elution**.
- These purified DNA fragments are used in constructing recombinant DNA by joining them with cloning vectors.

2. Cloning Vectors

- They are the DNA molecules that can carry a foreign DNA segment and replicate inside the host cells E.g. **Plasmids** (circular extra-chromosomal DNA of bacteria) and **bacteriophages**.
- Bacteriophages (high number per cell) have very high copy numbers of their genome within the bacterial cells.
- Some plasmids have only 1-2 copies per cell. Others may have 15-100 copies per cell.
- When the cloning vectors are multiplied in the host the linked piece of DNA is also multiplied to the numbers equal to the copy number of the vectors.

Features of cloning vector :

(a) Origin of replication (*ori*)

- This is a DNA sequence from where replication starts. A piece of DNA linked to *ori* site can replicate within the host cells. This also controls the copy number of the linked DNA. So, for getting many copies of the target DNA it should be cloned in a vector whose origin support high copy number.

(b) Selectable marker (marker gene)

- It helps to select the transformants and eliminate the non-transformants.
- **Transformation** is a procedure in which a piece of DNA is introduced in a host bacterium.
- Selectable markers of *E. coli* include the genes encoding resistance to antibiotics like ampicillin, chloramphenicol, tetracycline or kanamycin etc.
- The normal *E. coli* cells do not carry resistance against any of these antibiotics.

(c) Cloning sites

- In order to link the alien DNA, the vector needs very few recognition sites for restriction enzymes.
- Presence of more than one recognition sites generates several fragments, which complicates the gene cloning.
- The ligation of alien DNA is carried out at a restriction site present in one of the two **antibiotic resistance genes**.

  E.g. ligation of a foreign DNA at the Bam HI site of tetracycline resistance gene in the vector pBR322.
- The recombinant plasmids lose tetracycline resistance due to insertion of foreign DNA But, they can be selected out from non-recombinant ones by plating the transformants on **ampicillin** containing medium.

  Then, these transformants are transferred on **tetracycline** medium.
- The recombinants grow in ampicillin medium but not on tetracycline medium.

  But, non-recombinants will grow on the medium containing both the antibiotics.
- In this case, one antibiotic resistance gene helps to select the transformants, whereas the other antibiotic resistance gene gets inactivated due to insertion of alien DNA and helps in selection of recombinants.
- Selection of recombinants due to inactivation of antibiotics requires simultaneous plating on two plates having different antibiotics.
- Therefore, alternative selectable markers have developed to differentiate recombinants from non-recombinants on the basis of their ability to produce colour in the presence of a chromogenic substrate.
- A recombinant DNA is inserted within the coding sequence of an enzyme, α-galactosidase. So, the enzyme is inactivated. It is called **insertional inactivation**. Such colonies do not produce any colour. These are identified as recombinant colonies.
- If the plasmid in bacteria do not have any insert it gives blue coloured colonies in presence of chromogenic substrate.

(d) Vectors for cloning genes in plants and animals

- Genetic tools of some pathogens can be transformed into useful vectors for delivering genes to plants and animals. E.g. *Agrobacterium tumefaciens* (a pathogen of many dicot plants) can deliver a piece of DNA (T-DNA) to transform normal plant cells into a tumor.

- These tumor cells produce the chemicals required by the pathogen.
- The **tumor inducing (Ti) plasmid** of *A. tumefaciens* is modified into a cloning vector which is not pathogenic to the plants but is able to use the mechanisms to deliver genes of interest into plants.
- Retroviruses in animals can transform normal cells into **cancerous** cells. So, they are used to deliver desirable genes into animal cells.

3. Competent Host (For Transformation with Recombinant DNA)

- Competent cells are capable of up taking DNA from the surrounding. For the process of transformation bacterial cells are made competent, so that DNA can enter the cells.
- DNA is a hydrophilic molecule. So it cannot pass through cell membranes.
- To avoid this problem, bacterial cells are treated with a specific concentration of a divalent cations (*e.g.* calcium), so as to increase the pore size in the cell wall.
- So, DNA enters the bacterium through pores in cell wall. Such cells are incubated with recombinant DNA on ice.
- They are then placed briefly at 42°C (heat shock) and then put them back on ice. This enables the bacteria to take up the recombinant DNA.

Other methods to introduce alien DNA into host cells :

(a) Micro-injection : In this, recombinant DNA is directly injected into the nucleus of an animal cell.

(b) Biolistics (gene gun) method : In this, cells are bombarded with high velocity micro-particles of gold or tungsten coated with DNA. This method is suitable for plants.

(c) 'Disarmed pathogen' vectors : These vectors, when infect the cell, transfer the recombinant DNA into the host.

IMPORTANT DIAGRAMS

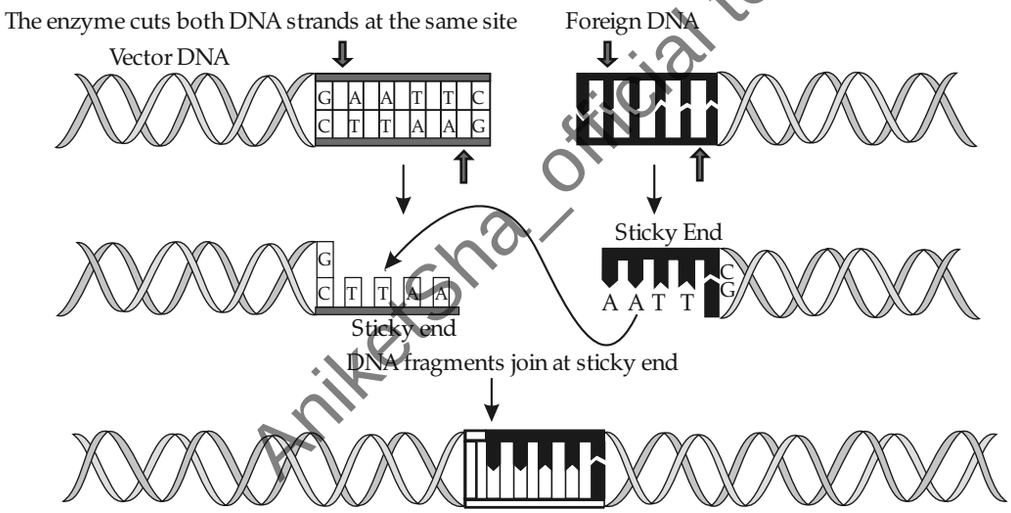


Fig 11.1: Steps in formation of recombinant DNA by action of restriction endonuclease enzyme- EcoRI.

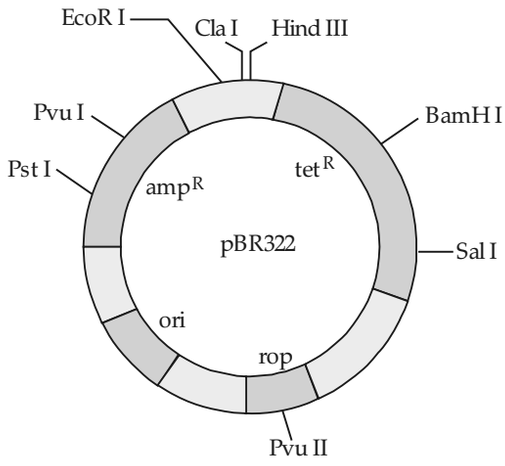


Fig 11.2: Cloning vector pBR322

Very Short Answer Type Questions (1 mark each)

Q.1. Suggest a technique to a researcher who needs to separate fragments of DNA.

R **[Delhi Set-I, 2016]**

Ans. Gel electrophoresis is used to separate DNA fragments.

[CBSE Marking Scheme, 2016] 1

Commonly Made Error

- Students often mis-spell the name of the techniques.

Q. 2. Name the technique that is used to alter the chemistry of genetic material (DNA, RNA) to obtain desired result.

R **[Delhi Set-I, II and III, Comptt., 2016]**

Ans. Genetic Engineering / Biochemical Engineering / Biotechnology. **[CBSE Marking Scheme, 2016] 1**

Q. 3. Mention the use of gel electrophoresis in biotechnology experiments.

A **[Outside Delhi Set-I, II and III, Comptt., 2016]**

Ans. Cut fragments of DNA can be segregated / separated. **1**

Q. 4. Name the material used as matrix in gel electrophoresis and mention its role.

R **[Outside Delhi, Set Comptt., 2014]**

Ans. Agarose is the most commonly used matrix in DNA gel electrophoresis. It provides sieving effect for separation of DNA fragments according to their size. **1**

Q. 5. Why it is not possible for an alien DNA to become part of a chromosome anywhere along its length and replicate normally ?

A **[Outside Delhi Set-III, 2014]**

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

[CBSE Marking Scheme, 2014] 1

Detailed Answer :

This is because the alien piece of DNA has to become part of a chromosome, which has the ability to replicate. In a chromosome there is a specific DNA sequence called the origin of replication, *Ori* which is responsible for initiating replication. **1**

Q. 6. Name the host cells in which micro–injection technique is used to introduce an alien DNA.

R **[Delhi Set-II, III, 2014]**

Ans. Animal cell.

[CBSE Marking Scheme, 2014] 1

Q. 7. Write the two components of the first artificial recombinant DNA molecule constructed by Cohen and Boyer. R **[Delhi Set-II, 2014]**

Ans. Restriction enzyme and vector. ½ + ½

Q. 8. Mention the type of host cells suitable for the gene guns to introduce an alien DNA.

R **[Delhi Set-I, III, 2014]**

Ans. Plant cells. **1**

Q. 9. State what happens when an alien gene is ligated at the Sal I site of pBR322 plasmid.

A **[Delhi Set-I, 2013]**

Ans. When an alien gene is ligated at the Sal I site of tetracycline resistance gene in the vector pBR322, the recombinant lose tetracycline resistance due to insertion of the foreign DNA. **1**

[CBSE Marking Scheme, 2013]

Q. 10. State what happens when an alien gene is ligated at *Pvu* I site of pBR322 plasmid.

R **[Outside Delhi Set-I, II, Comptt., 2013]**

Ans. When an alien gene is ligated at the Pvu I site of ampicillin resistance gene in the vector pBR322, the recombinant plasmids lose ampicillin resistance due to insertion of the foreign DNA.

[CBSE Marking Scheme, 2013] 1

AI **Q. 11. Why is 'plasmid' an important tool in bio-technology experiments ?**

U **[Outside Delhi Set-II, Comptt., 2013]**

Ans. Plasmids are commonly used to multiply or express particular genes and act as vectors to transfer piece of foreign DNA attached to them.

[CBSE Marking Scheme, 2013] 1

Q. 12. How is the action of normal endonuclease enzymes different from that of restriction endonuclease ?

U **[CBSE SQP, 2013]**

[Delhi Set-I, Comptt. 2012]

Ans. Normal endonuclease cuts at random position within a DNA sequence, whereas restriction endonuclease recognizes and cut specific nucleotide sequences within DNA. **1**

Q. 13. Which main technique and instrument is used to isolate DNA from a plant cell ?

R **[CBSE SQP, 2013; Delhi Set-I, Comptt., 2012]**

Ans. Centrifugation and centrifuge. **1**

Q. 14. Why EtBr is used in gel electrophoresis in spite of it being highly carcinogenic ?

R **[CBSE SQP, 2013]**

Ans. EtBr is an intercalating agent. It stacks itself in the DNA bases, and fluoresce under U.V light, thus helps in identification of DNA.

[CBSE Marking Scheme, 2013] 1

AI **Q. 15. Why do DNA fragments move towards the anode during gel electrophoresis ?**

R **[Delhi/Outside Delhi, Comptt, Set 1,2,3, 2018, 2011]**

Ans. DNA fragments are negatively charged. **1**
[CBSE Marking Scheme, 2018]

Detailed Answer:

In gel electrophoresis, DNA fragments are negatively charged molecules. They can be separated by forcing them to move towards the anode under electric field through a medium / matrix.

Q. 16. Why is it essential to have 'selectable marker' in cloning vector.
R **[Outside Delhi 2010, Delhi Set, 2011]**

Ans. Selectable marker helps in the identification and elimination of non-transformants and permitting the growth of the transformants. Therefore, they are considered essential in cloning vector. **1**

Q. 17. Write the palindromic sequence that EcoRI recognises.
R **[Delhi Comptt. - 2017, Set - II]**

Ans. Palindromic sequence is asked, write both the sequence with polarity.
5′-GAATTC-3′
3′-CTTAAG-5′ **1**
[CBSE Marking Scheme, 2017]

Commonly Made Error
- Students often write incorrect palindromic sequence.

Answering Tip
- Carefully understand the concept of palindromic sequences. Stress on learning the palindromic sequence of various restriction enzymes.

Q. 18. Name two enzymes that are essential for constructing a recombinant DNA.
R **[Delhi Comptt. - 2017, Set - II]**

Ans. Restriction enzymes / polymerase enzymes / ligase **(Any two)** ½ + ½
[CBSE Marking Scheme, 2017]

Q. 19. Mention the role of Restriction Enzymes in Recombinant DNA technology.
R **[Delhi Comptt. - 2017, Set - I]**

Ans. To cut DNA at specific sites / Molecular scissors (DNA). **[CBSE Marking Scheme, 2017]** **1**

Q. 20. Write the two components of the first artificial recombinant DNA molecule constructed by Cohen and Boyer. R **[SQP, 2017]**

Ans. The two components are antibiotic resistant gene and plasmid vector of *Salmonella typhimurium*. **1**
[CBSE Marking Scheme, 2017]

Q. 21. Name the specific sequence of DNA in a plasmid that the gene of interest ligates with to enable it to replicate. R **[Outside Delhi, Comptt., 2013]**

Ans Origin of replication (Ori). **1**

AI **Q. 22. How can following be made possible for biotechnology experiments?**
(i) Isolation of DNA from bacterial cell.
(ii) Reintroduction of recombinant DNA into a bacterial cell. R **[Foreign 2012]**

Ans. (i) By treating the cell with the enzyme lysozyme.
(ii) By making the bacterial cell competent. **1**

Q. 23. Explain giving reasons why an alien piece of DNA needs to be integrated to a specific sequence of host DNA for its cloning ?
A **[Outside Delhi Comptt., 2011]**

Ans. Ori (origin of replication) is the specific DNA sequence where the replication of DNA is initiated. Therefore for multiplication of alien DNA in the host it has to be integrated to the ori (origin of replication). **1**

Short Answer Type Questions-I **(2 marks each)**

Q. 1. Explain palindromic nucleotide sequence with the help of a suitable example.
R **[Delhi Set-III, 2014]**

Ans. Palindrome in DNA is a sequence of base pairs that reads the same on two strands when orientation of reading is the same.
Example : 5′ —— GAATTC —— 3′
3′—— CTTAAG —— 5′ **2**
[CBSE Marking Scheme, 2014]

Q. 2. Why are molecular scissors so called ? Write their use in biotechnology. R **[Delhi Set-II, 2014]**

Ans. The restriction enzymes are known as molecular scissors as they cut the DNA at specific sites or locations.
They help (in genetic engineering) to form recombinant molecules of DNA, which are composed of DNA from different genomes.
1 + ½ + ½
[CBSE Marking Scheme, 2014]

Commonly Made Error
- Students often write vague answers.

AI **Q. 3. Explain with the help of a suitable example the naming of a restriction endonuclease.**
U **[Delhi Set-I, 2014]**

Ans. EcoRI.
The first letter of the name comes from the genus and the next two from the name of the species of the bacterium *i.e.* prokaryotic cell. Thus *Eco* stands for the genus and species of the prokaryotic cell from which the enzyme was isolated *i.e. E. coli*
R stands for strain.
'I' follows order in which enzyme was isolated. **2**
[CBSE Marking Scheme, 2014]

Answering Tip
- Rules of naming restriction endonuclease should be learned thoroughly.

Q. 4. How does a restriction nuclease function ? Explain. R **[Outside Delhi Set-III, 2014]**

Ans. Restriction nuclease cut DNA at specific sites. Exonuclease cuts DNA at the ends, endonuclease cuts at specific position within DNA. **1**

Restriction endonuclease cuts the DNA at specific palindromic sequence. **1**

[CBSE Marking Scheme, 2014]

Commonly Made Error

- Students get confused between restriction enzymes like endonuclease and exonuclease.

Answering Tip

- Learn the differences between endonuclease and exonuclease enzymes with the help of diagram for better retention and understanding.

Q. 5. How are 'sticky ends' formed on a DNA strand ? Why are they so called ? R **[Delhi Set-II, 2014]**

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. **½ × 3 = 1½**

These overhang stretches are called as sticky ends. They form hydrogen bonds with their complementary cut counterparts. **½**

[CBSE Marking Scheme, 2014]

AI **Q. 6. Write the role of 'Ori' and 'restriction site in a cloning vector pBR322.** R **[Delhi Set-II, 2014]**

Ans. **Ori :** It is a genetic sequence that acts as the initiation site for replication of DNA. Any fragment of DNA, when linked to the ori region, can be initiated to replicate.

Restriction site : It is the recognition site for restriction enzymes (such as EcoRI, Hind III, Pvu I and Bam HI). Recognition sites are the genetic sequences from where the restriction enzymes cut the DNA. **1 + 1 = 2**

[CBSE Marking Scheme, 2014]

Q. 7. Explain how to find whether *E. coli* bacterium has transformed or not, when, a recombinant DNA bearing ampicillin-resistant gene is transferred into it. R **[Delhi Set-II, 2013]**

Ans. The recombinant / transformant may be found out from non-recombinant / non-transformant by plating the transformants on ampicillin containing medium. The transformants growing on ampicillin containing medium are then transferred to tetracycline containing medium. The recombinant will grow on ampicillin containing medium but not on that containing tetracycline. But non-recombinant will grow on both tetracycline and ampicillin containing media. **2**

Q. 8. Explain the work carried out by Cohen and Boyer that contributed immensely to biotechnology.

R **[Outside Delhi Set-III, 2012]**

Ans. Cohen and Boyer invented the technique of DNA Cloning and conducted first genetic engineering experiments. They conducted experiment on removing plasmid from one bacterial cell and reinserting into another bacterial cell.

[CBSE Marking Scheme, 2012] 2

Q. 9. List the key tools used in recombinant DNA technology. R **[Delhi Set-I, 2011]**

Ans. Restriction enzymes / Polymerase enzymes / Ligase enzymes / Vectors / Host organisims / *E. coli*/ *Agrobacterium*. [CBSE Marking Scheme, 2011] 2

Q. 10. State the role of DNA ligase in biotechnology.

R **[Delhi Set-I, II, III, 2011]**

Ans. The role of DNA ligase in biotechnology is to join two different restricted fragments of DNA to make recombinant DNA. the DNA fragments are joined from their ends. **2**

Q. 11. What is EcoRI ? How does EcoRI differ from an exonuclease ? R **[Delhi Set-I, Comptt. 2015]**

Ans. EcoRI is restriction endonuclease enzyme. **1**

Exonuclease removes nucleotides from the ends of DNA. **½**

EcoRI makes cuts at specific position within the DNA. **[CBSE Marking Scheme, 2015] ½**

Answering Tip

- Learn the differences between exonuclease and endonuclease with examples.

Q. 12. State how has *Agrobacterium tumefaciens* been made a useful cloning vector to transfer DNA to plant cells. R **[Delhi Set-I, 2011]**

Ans. *Agrobacterium tumefaciens* has Ti plasmid. This plasmid is modified into a cloning vector, which is no more pathogenic to host plants and is able to deliver genes of interest. **½ × 4 = 2**

Q. 13. Explain the role of Ti plasmid in biotechnology.

R **[Delhi Set-I, II, III, 2011]**

Ans. (i) The Ti plasmid (tumor-inducing plasmid) of *Agrobacterium tumefaciens* has been modified (does not cause tumour) and used as a cloning vector. The Ti plasmid integrates a segment of its DNA, termed T-DNA into the chromosomal DNA of its host plant cells.

(ii) The T-DNA plasmid causes tumours. As gene transfer occurs without human effort, the bacterium is known as 'natural genetic engineer' of plants. Ti plasmids as vectors, transfer foreign genes of interest into the target cells. **1 + 1 = 2**

Short Answer Type Questions-II (3 marks each)

Q 1. Describe a palindrome with the help of an example. R **[Delhi Set-II, Comptt., 2016]**

Ans. A DNA sequence that reads the same, on the two strands from 5′- 3′ direction or 3' → 5' direction. **1**

5′-GAATTC-3′
3′-CTTAAG-5′ **1 + 1**

[CBSE Marking Scheme, 2016]

Answering Tip

- Lay stress on the importance of directionality.

AI **Q. 2. (i) Name the selectable markers in the cloning vector pBR322. Mention the role they play.**

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

R **[Outside Delhi Set-I, 2016]**

Ans. (i) amp^R / ampicillin resistance genes, tet^R/ tetracycline resistance gene. $\frac{1}{2} \times 2 = 1$

They help in identifying and eliminating non-transformants / non- recombinants and selectively permitting the growth of the transformants / recombinants.

(ii) Simpler process / less cumbersome, in the presence of chromogenic substrate recombinants are colourless and non recombinants are blue in colour. **2**

[CBSE Marking Scheme, 2016]

Detailed Answer :

(i) In cloning vector pBR322, ampicillin and tetracycline resistance genes serve as selectable markers. Selectable markers help in the identification and selection of transformed cells from non-transformed cells to distinguish the recombinant cells from the non-recombinant cells.

(ii) The coding sequence of an enzyme β-galactosidase is preferred over antibiotic resistance genes because recombinants can be easily visualised and the process is comparatively simple and less cumbersome. When the foreign gene is inserted within the β-galactosidase gene, the enzyme β-galactosidase gets inactivated (insertional inactivation). Thus, when the bacteria are grown on a chromogenic substrate, non-recombinants will produce blue-coloured colonies while the recombinants will produce colourless colonies.

OR

(a) Selectable markers in pBR322 are — in amp^R & tet^R.
Selectable markers play an important role in a way that enables us to seperate (select) transformants from non transformants and recombinants from non recombinant organisms.

(b) The use of β-galactosidase gene as a selectable marker makes the job of selection easy as transformants and recombinants can be seperated on a single plating. The Recombinants dont give any colour, while non recombinants provide blue colour in presence of chromogenic substrate.
But while using amp^R and tet^R, two platings are required, one for selecting transformants and other for selecting recombinants which is a cumbersome procedure.

[Topper's Answer, 2016]

Commonly Made Error

- Most of the students get confused between recombinants and non-recombinants. Many of them write that the *recombinant colonies turn blue and non-recombinant colonies remain white,* which was just the opposite of the correct answer.

Q. 3. (i) Why must a cell be made 'competent' in biotechnology experiments ? How does calcium ion help in doing so ?

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

R **[Outside Delhi Set-I, 2016]**

Ans. **(i)(a)** To take up the (hydrophilic) DNA from the external medium. **1**

(b) Divalent calcium ions increase the efficiency of the cell to take up foreign DNA through pores in the cell wall. **1**

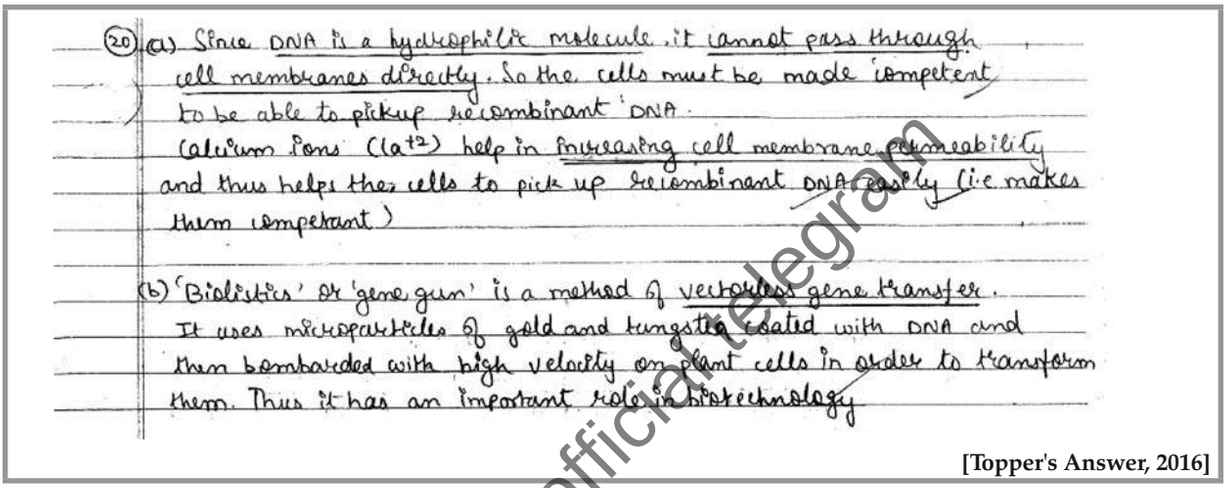
(ii) To introduce alien DNA into the plant cell by bombarding them with high velocity microparticles (gold or tungsten coated with DNA). **[CBSE Marking Scheme, 2016] 1**

Detailed Answer :

(i) In biotechnology experiments, the cells must be made competent so that they can take up the hydrophilic DNA molecule inside them from the external medium. Treatment of bacterial cells with divalent calcium cations makes them competent and helps them to take up the DNA through the pores in the cell wall.

(ii) Biolistic gene or gene gun is a method of introducing alien DNA into the plants cells. In this method, the host cells are bombarded with high velocity micro-particles of gold or tungsten coated with DNA molecules.

OR


(20) (a) Since DNA is a hydrophilic molecule, it cannot pass through cell membranes directly. So the cells must be made competent to be able to pickup recombinant DNA.
Calcium ions (Ca^{+2}) help in increasing cell membrane permeability and thus helps the cells to pick up recombinant DNA easily (i.e makes them competant)

(b) 'Biolistics' or 'gene gun' is a method of vectorless gene transfer. It uses microparticles of gold and tungsten coated with DNA and then bombarded with high velocity on plant cells in order to transform them. Thus it has an important role in biotechnology

[Topper's Answer, 2016]

Q. 4. (i) 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 :

PvuI, EcoRI, BamHI

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

A **[CBSE SQP 2016-17]**

Ans. **(i)** Bam HI should be used, as restriction site for this enzyme is present in tet^R region.

(ii) Pvu I will not be used as restriction site for this enzyme is present in amp^R region (not in tet^R).

EcoRI will not be used, as restriction site for this enzyme is not present in selectable marker tet^R . **3**

[CBSE Marking Scheme, 2016]

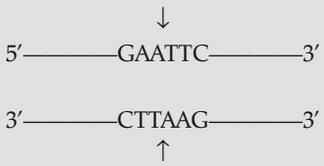
Answering Tip

- Carefully learn the role of cloning sites in a cloning vector. Keywords should be highlighted in the answers.

Q. 5. Explain with the help of an example the relationship between restriction endonuclease and a palindromic nucleotide sequence.

R **[Foreign Set-II, 2016]**

Ans. Restriction endonuclease recognises a specific palindromic nucleotide sequence, in the DNA molecule. 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. **½×4=2**



1

(No mark, if polarity is not shown or if only one strand is shown).

[CBSE Marking Scheme, 2016]

Commonly Made Error

- Students often forget to mark the polarity of sequence. If polarity is not shown or if only one strand is shown, this may deduct your marks.

Answering Tip

- Lay stress on the importance of directionality.

Q. 6. How does a restriction endonuclease help in DNA recombinant technology ?

R **[Foreign Set-III, 2016]**

OR

Explain the mode of action of EcoRI.

[Delhi Set-III, Comptt. 2016]

Ans. Restriction endonuclease (EcoRI) inspects length of DNA and recognises specific palindromic nucleotide sequence, binds with DNA, cuts each of the two strands of double helix at specific points.

This leaves the single stranded overhanging stretches at the ends. They are called sticky ends. They form H-bonds with their complementary cut counterparts. This stickiness facilitates action of DNA ligase, when cut by the same restriction enzyme. The resultant DNA fragments have the same kind of sticky ends and these are joined together by DNA ligase. **1×3=3**

[CBSE Marking Scheme, 2016]

Q. 7. Explain the role of the enzyme EcoRI in recombinant DNA technology.

R **[Foreign Set-I, 2016]**

Ans. EcoRI (Restriction endonuclease) acts as molecular scissors. They serve as tools for cutting DNA molecule at specific palindromic sites, inspects length of DNA and recognises specific palindromic nucleotide sequence, binds with DNA, cuts each of the two strands of double helix at specific points. **3**

Q. 8. (i) Differentiate between exons and introns.

(ii) What is a plasmid ? Why is it selected as a vector ?

R **[Outside Delhi Set-I, Comptt., 2015]**

Ans. (i) Exons are the coding or expressed sequences that appear in mature or processed RNA, introns are intervening sequences that do not appear in mature or processed RNA / Exons are codons that code for amino acid sequence, introns do not code for amino acids. **1**

(ii) Autonomously replicating circular DNA / extra chromosomal DNA, exclusively present in bacteria. ½ + ½

Plasmid is selected as vector because it has ability to replicate in the bacterial cell independent of chromosomal DNA and also has high copy number. ½ + ½

AI **Q. 9. (i) Draw the figure of vector pBR322 and label the following :**

Origin of replication

Ampicillin resistance site

Tetracycline resistance site

Bam H1 restriction site.

(ii) Identify the significance of origin of replication.

R **[CBSE SQP, 2015]**

Ans. (i) For diagram: Refer Topic 1/ Revision Notes/ Important Diagrams/ Fig 11.2 ½ +4

(ii) Origin of replication is responsible for controlling the copy number of the DNA sequence inserted. **1**

[CBSE Marking Scheme, 2015]

Answering Tip

- Carefully draw the diagram and label the asked parts carefully. Each labeling carries a mark.

Q. 10. Name and describe the technique that helps in separating the DNA fragments formed by the use of restriction endonuclease.

R **[Outside Delhi Set-III, 2014]**

Ans. Gel electrophoresis.

DNA are negatively charged forced to move towards anode, electric field in agarose gel matrix, separate according to their size / sieving effect, smaller fragments moves faster and farther than the larger. **1 + 2**

[CBSE Marking Scheme, 2014]

Detailed Answer :

Gel electrophoresis, Since DNA fragments are negatively charged molecules, they can be separated by forcing them to move towards the anode under an electric field through a medium / matrix. The most commonly used matrix is agarose which is a natural polymer extracted from sea weeds.

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. **3**

Q. 11. Explain the basis on which the gel electrophoresis technique works. Write any two ways the products obtained through this technique can be utilized.

A **[Delhi Set-I, Comptt., 2014]**

Ans. Basis of technique : Since DNA fragments are negatively charged, they can be separated by forcing them to move towards the anode under an electric field through a medium of matrix (agarose).

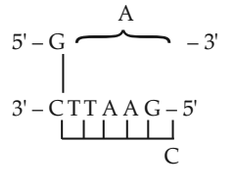
Uses : (i) The purified DNA fragments are obtained by gel electrophoresis.

(ii) They are used in DNA fingerprinting. **3**

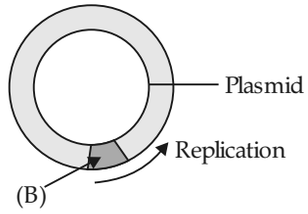
[CBSE Marking Scheme, 2014]

Q. 12. (i) Identify (A) and (B) illustrations in the following :

(a)



(b)



(ii) Write the term given to (A) and (B) and why ?

(iii) Expand PCR. Mention its importance in biotechnology.

R **[Delhi Set-I, II, III, 2011, KVS, DDE]**

Ans. (i) (A)–AATTC / Sticky end.
(B)–*Ori* / Origin of Replication.

(ii) Palindromic sequence, because the sequence of base pair reads same on the two strands, when orientation of reading is kept the same.

(iii) **PCR** – Polymerase Chain Reaction.
Importance – amplification of gene of interest (*in vitro*). **[CBSE Marking Scheme, 2011] 3**

Detailed Answer :

(i) The part labelled A is the sticky end.

The part labelled B is the foreign DNA insert. The part labelled C is the *Ori* or Origin of Replication.

(ii) The term used for A and C are called the palindromic nucleotide sequence. These are named so because they read the same forward and backward.

(iii) PCR stands for Polymerase Chain Reaction.

PCR is a technique in molecular biology, used to amplify a gene or a piece of DNA to obtain its several copies. It is extensively used in the process of gene manipulation. **3**

Q. 13. How are the following used in biotechnology ?

(i) plasmid-DNA.

(ii) recognition sequence.

(iii) gel electrophoresis.

R **[Outside Delhi Comptt., 2011]**

Ans. (i) Plasmids are relatively small DNA sequences that can self replicate and exist independent of the chromosome. Plasmids often carry antibiotic resistance genes that makes them selectable. They can be genetically modified - cut at specific locations using restriction enzymes and new DNA sequences are included. This recombinant plasmid is reintroduced into bacteria and easily forced to multiply in large numbers at relatively low cost.

Thus, plasmids have been used in biotechnology to develop vectors (tools) for basic research like studying new genes as well as to produce therapeutic chemicals.

(ii) The restriction enzymes cut the DNA at a specific point called recognition sequence or sites. Each restriction enzyme has its particular restriction site. Thus, recognition sequences play a vital role in giving direction to the restriction enzymes regarding the particular position of cleavage in the DNA.

(iii) Gel electrophoresis is a technique used to separate DNA fragments according to their size. DNA fragments are negatively charged, so they move towards the positive electrode. Because all DNA fragments have the same amount of charge per mass, small fragments move through the gel faster than larger ones.

When a gel is stained with a DNA-binding dye, the DNA fragments can be seen as bands, each representing a group of same-sized DNA fragments. **1 × 3 = 3**

Q. 14. Explain the roles of the following with the help of an example each in recombinant DNA technology:

(a) Restriction Enzymes

(b) Plasmids

R **[Delhi/Outside Delhi, 2018]**

Ans. (a) It recognizes a specific sequence of base pairs palindromes and cuts the DNA strand at a specific site. ½ + ½
E.g. EcoRI / Hind II or any other correct example. ½

(b) Act as vectors / cloning of desired alien gene / foreign gene. **1**
E.g. pBR322 / plasmid of *Salmonella* / plasmid of *Agrobacterium* / Ti plasmid/ Tumour inducing plasmid. ½

[CBSE Marking Scheme, 2018]

Answering Tip

- Learn the concept of recombinant DNA technology carefully. Don't forget to learn the role of tools used in it.

Q. 15. 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.

R **[Delhi Set-I, 2010]**

Ans. For diagram: Refer Topic 1/ Revision Notes/ Important Diagram/ Fig 11.1

[CBSE Marking Scheme, 2010] 3

Q. 16. (i) Explain the significance of 'palindromic nucleotide sequence' in the formation of recombinant DNA.

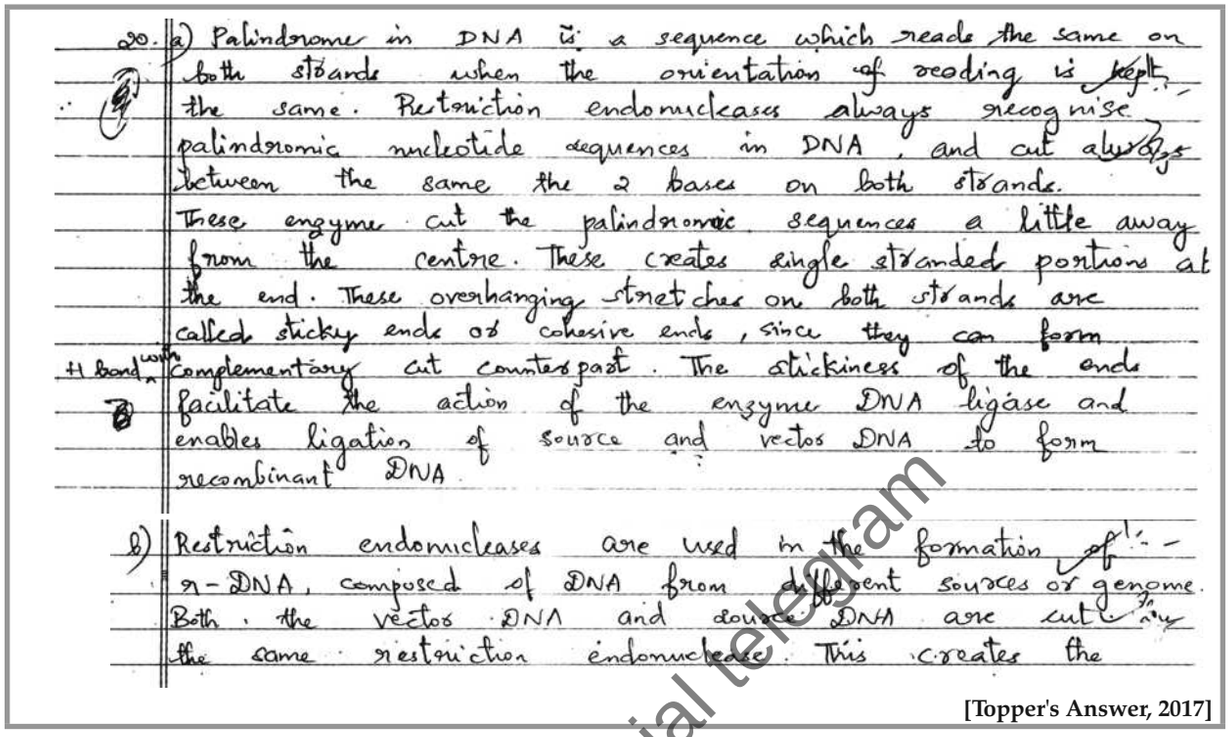
(ii) Write the use of restriction endonuclease in the above process.

U **[Outside Delhi, 2017, Set - I, II, III,]**

Ans. (i) Palindromic nucleotide sequence is the recognition (specific) sequence present both on the vector and on a desired / alien DNA for the action of the same (specific) restriction endonuclease to act upon. **2**

(ii) Same restriction endonuclease binds to both the vector and the foreign DNA, cut each of the two strands of the double helix at specific points in their sugar phosphate backbone of recognition sequence for restriction endonucleases / palindromic sequence of vector and foreign DNA, to cut strand a little away from the centre of the palindrome sites, creates overhanging stretches / sticky ends. ½ × **2**

OR



20. a) Palindrome in DNA is a sequence which reads the same on both strands when the orientation of reading is kept the same. Restriction endonucleases always recognise palindromic nucleotide sequences in DNA, and cut always between the same the 2 bases on both strands. These enzyme cut the palindromic sequences a little away from the centre. These creates single stranded portions at the end. These overhanging stretches on both strands are called sticky ends or cohesive ends, since they can form +1 bond with complementary cut counterpart. The stickiness of the ends facilitate the action of the enzyme DNA ligase and enables ligation of source and vector DNA to form recombinant DNA

b) Restriction endonucleases are used in the formation of r-DNA, composed of DNA from different sources or genome. Both the vector DNA and source DNA are cut by the same restriction endonuclease. This creates the

[Topper's Answer, 2017]

AI **Q. 17. Explain the role(s) of the following in Biotechnology :** U **[Delhi - 2017, Set - I, II, III]**

(i) Restriction endonuclease

(ii) Gel - electrophoresis

(iii) Selectable markers in pBR322.

Ans. (i) Cuts at specific position within the DNA / cuts DNA at specific nucleotide / cuts at palindromic nucleotide sequence. **1**

(ii) Separation of DNA fragments (under the influence of electric field). **1**

(iii) Helps in identifying and eliminating non-transformants from transformants / selection of transformants. **1**

[CBSE Marking Scheme, 2017]

Detailed Answer.

(i) The enzyme restriction endonuclease recognise the base sequence at palindrome sites in DNA and cut it's strand.

(ii) Gel-electrophoresis is a technique of separation of charged molecules under the influence of an electric field. With the help of gel electrophoresis technique, DNA fragments get separated according to size through the pores of agarose gel.

(iii) Selectable marker (marker gene) helps to select the transformants and eliminate the non-transformants.

Q. 18. Name and explain the technique that helps in the separation of DNA fragments for DNA recombinant technology experiments. How can these separated DNA fragments be visualised ? R **[Delhi - 2017, Set - II]**

Ans. Gel electrophoresis, Since DNA fragments are negatively charged, they move towards anode (under an electric field) through a medium / matrix / agarose gel. The fragments separate (resolve) according to their size through sieving effect provided by agarose gel. The separated DNA fragments can be visualised after staining the DNA with ethidium bromide, followed by exposure to UV radiation. **3**

[CBSE Marking Scheme, 2017]

Detailed Answer :

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

DNA fragments separate according to their size through the pores of agarose gel.

The separated DNA fragments can be seen only after staining the DNA with a compound known as ethidium bromide (Et + Br) followed by exposure to UV radiation as bright orange colored bands.

Q. 19. (i) Why must bacterial cells be first made 'competent' in r-DNA technology ? How is this process carried out? [KVS]

(ii) Name the method by which an alien DNA can be made to enter (a) plant cell; (b) animal cell.

A **[Outside Delhi Comptt. 2017, Set - III]**

Ans. **(i)** Since DNA is hydrophilic, it cannot pass through cell membrane, hence bacterial cells are made competent. ½ + ½

By treatment with a specific concentration of a divalent cations, such as Ca^{++} which increases efficiency of entry of DNA through the pores of cell wall. **1**

(ii) **(a)** Plant cells - biolistic / gene guns ½

(b) Animal cells - Micro injection ½

[CBSE Marking Scheme, 2017]

Detailed Answer:

(i) DNA is a hydrophilic molecule, so it cannot pass through cell membrane. To avoid this problem, bacterial cells are treated with a specific concentration of a divalent cations (e.g., calcium), so as to increase the pore size in the cell wall. As a result, DNA enters the bacterium through pores of cell wall.

(ii) Other methods to introduce alien DNA into host cell are :

(a) Plant cell : Biolistics (gene gun) method.

(b) Animal cell : Micro - injection.

Q. 20. Mention the role of (i) selectable marker, (ii) Ori and (iii) rop in *E. coli* cloning vector pBR322.

U **[Outside Delhi Comptt. 2017, Set - II, III]**

Ans. **(i)** **Selectable marker :** Helps in identifying and eliminating non transformants and selectively permitting the growth of the transformants. **1**

(ii) **Ori :** Helps to start replication and any piece of DNA when linked to this sequence can be made to replicate within host cell, responsible for controlling the copy number of the linked DNA. ½ + ½

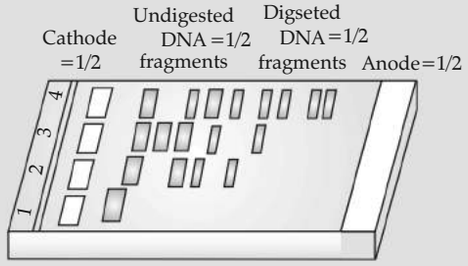
(iii) Codes for the proteins involved in the replication of the plasmid. **1**

[CBSE Marking Scheme, 2017]

Q. 21. Draw a diagram of a typical agarose gel electrophoresis showing migration of undigested and digested sets of DNA fragments. Label (a) the digested and undigested DNA fragments, (b) Anode and cathod ends of the plate. Mention the role of electrophoresis in biotechnology.

U **[Delhi - 2017, Set - I]**

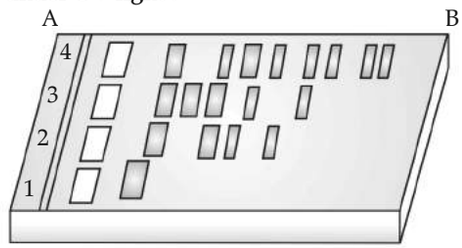
Ans.



The cutting of DNA by restiction endonuclease results in fragments of DNA. These fragments can then be separated by (Gel) electrophoresis. **2 + 1**

[CBSE Marking Scheme, 2017]

Q. 22. Given below is the diagram of agarose gel kept under UV light :



(i) Mark the positive and negative terminals.

(ii) What is the charge carried by DNA molecule.

(iii) How are the separated DNA fragments finally isolated ? A **[SQP, 2016-17]**

Ans. **(i)** Positive terminal-'B'

Negative terminal-'A'

(ii) DNA being negatively charged, moves towards the positive electrode (anode).

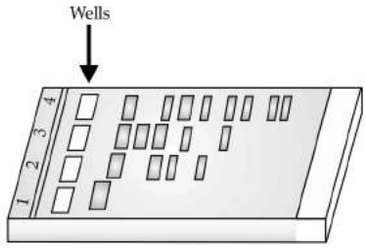
(iii) By elution-separated bands of DNA are cut out from the agarose gel and extracted from the gel piece.

[CBSE Marking Scheme, 2016] 3

Q. 23. (a) How do DNA fragments migrate and resolve in a Gel electrophoresis?

(b) How lane one is different from lane 2, 3 and 4 in the Gel electrophoresis set up?

(c) How pure DNA fragments are made observable in the visible light?



E & A **[CBSE SQP, 2018]**

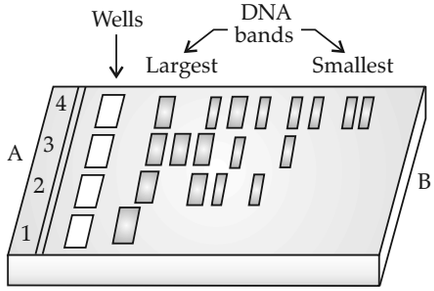
Ans. **(a)** The DNA fragments resolve according to their size through sieving effect provided by the agarose gel. Hence, the smaller the fragment size, the farther it moves. **1**

(b) The given agarose gel electrophoresis shows migration of undigested DNA fragments in lane 1 and digested set of DNA fragments in lane 2 to 4. **1**

(c) The separated DNA fragments can be visualized only after staining the DNA with a compound known as ethidium bromide followed by exposure to UV radiation. **1**

[CBSE Marking Scheme, 2018]

Q. 24. Rajesh was doing gel electrophoresis to purify DNA fragments. Given below is the sketch of the observations of the experiment performed by him.



(i) At which end he would have loaded the samples and where ?

(ii) Analyse the reason for different positions taken up by the DNA bands.

(iii) Elaborate the step he would have followed to visualize DNA bands. R **[CBSE SQP, 2015]**

Ans. (i) He would have loaded the samples near end A, in the wells.

(ii) The DNA fragments separate (resolve) according to their size through sieving effect provided by the agarose gel. Hence, the smaller is the fragment size, the farther it moves.

(iii) After staining the DNA with ethidium bromide followed by exposure to UV radiations, the DNA bands fluoresce.

[CBSE Marking Scheme, 2015] 1+1+1

Long Answer Type Questions

(5 marks each)

Q. 1. Unless the vector and source DNA are cut, fragments separated and joined, the desired recombinant vector molecule cannot be created.

(i) How are the desirable DNA sequence cut ?

(ii) Explain the technique used to separate the cut fragments.

(iii) How are the resultant fragments joined to the vector DNA molecule?

R **[Delhi Set-I, Comptt., 2015]**

Ans. (i) DNA sequences of the vector as well as the source are cut by the same restriction enzyme like EcoRI, in a palindromic sequence.

(The cut ends overhang as sticky ends in the medium.)

(ii) These cut ends fragments are to be extracted from the culture medium using gel electrophoresis.

This has an agarose gel matrix. Fragments are fed in the wells. DNA are negatively charged so, they move towards anode under an electric field through the gel. Smaller fragments move faster, thus separated.

(iii) Fragments are now added to the medium containing the vector DNA.

The sticky ends facilitates the action of the enzyme ligase and join the source DNA to the vector. **5**

AI **Q. 2. (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 ?

U **[Delhi Set-I, 2011]**

Ans. (i) (a) Should have *ori* / origin of replication, Has selectable marker, genes encoding for an antibiotic resistance / genes encoding for α-galactosidase **(b)** Has cloning site / recognition site, for the restriction enzyme to recognise.

(ii) DNA is a hydrophilic molecule.

Bacterial cell is made competent by treating with specific concentration of Ca^{++} ions / divalent ions, incubating them on ice, heat shock for a short period and placing it back once again. **2½ + 2½=5**

[CBSE Marking Scheme, 2011]

Detailed Answer :

(i) The following are the features that are required to facilitate cloning into a vector :

(a) Origin of replication (*ori*) : This 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. This sequence is also responsible for controlling the copy number of the linked DNA.

(b) Selectable marker : The vector requires a selectable marker, which helps in identifying and eliminating non transformants and selectively permitting the growth of the transformants. **Transformation** is a procedure through which a piece of DNA is introduced in a host bacterium. Normally, the genes encoding resistance to antibiotics such as ampicillin, chloramphenicol, tetracycline or kanamycin, etc. are considered useful selectable markers for *E. coli*.

(c) Cloning sites : In order to link the alien DNA, the vector needs to have very few **recognition sites** for the commonly used restriction enzymes. Presence of more than one recognition sites within the vector will generate several fragments, which will complicate the gene cloning. **5**

TOPIC-2
Process of Recombinant DNA Technology

Revision Notes

1. **Isolation of the Genetic Material (DNA)**
   - To get pure DNA (free from other macro-molecules), the bacterial cells / plant or animal tissue are treated with enzymes such as **lysozyme** (bacteria), **cellulase** (plant cells), **chitinase** (fungus), etc.
   - The cell is broken to release DNA along with other macro-molecules (RNA,proteins, polysaccharides and lipids).
   - Genes (DNA) are interwined with proteins such as histones.
   - RNA is removed by treating with **ribonuclease**.
   - Proteins are removed by treatment with **protease**.
   - Other molecules are removed by appropriate treatments.
   - When chilled ethanol is added, purified DNA precipitates out as a collection of fine threads in the suspension.
2. **Cutting of DNA at Specific Locations**
   - Restriction enzyme digestions are performed by incubating purified DNA with the restriction enzyme, at the optimal conditions.
3. **Isolation of derived DNA fragments.**
   - **Agarose gel electrophoresis** is employed to check the progression of a restriction enzyme digestion. As DNA is negatively charged, it moves towards the anode. The process is repeated with the vector DNA also.
   - After cutting the source DNA and the vector DNA, the cut out gene (DNA segment) of interest from the source DNA and the cut vector are mixed and ligase is added.
   - This creates recombinant DNA.
4. **Amplification of Gene of Interest Using PCR**
   - **Polymerase Chain Reaction (PCR)** is the synthesis of multiple copies of the gene of interest *in vitro* using two sets of **primers** and the enzyme **DNA polymerase**.
   - The technique was developed by *Kary Mullis* in 1985 and for this he was awarded the Nobel Prize in 1993.
   - Primers are small chemically synthesized oligonucleotides that are complementary to the regions of DNA.
   - The enzyme extends the primers using the nucleotides and the genomic DNA (template).
   - For amplification a thermostable DNA polymerase (isolated from a Thermophilic bacterium, *Thermus aquaticus*) is used.
   - It remains active at high temperature during the denaturation of double stranded DNA.
   - Source DNA and vector DNA are cut with the same endonuclease so as to obtain the sticky ends.
   - These are then ligated by mixing the gene of interest vector DNA in presence of the enzyme DNA ligase to form recombinant DNA.
   - The amplified fragment can be used to ligate with a vector for further cloning.
5. **Insertion of Recombinant DNA into the Host Cell / organism**
   - There are several methods of introducing the ligated DNA into recipient cells.
   - Recipient cells take up DNA present in its surrounding.
   - If a recombinant DNA bearing **ampicillin resistant gene** (a selectable marker gene) is transferred into ***E. coli*** cells,the host cells become ampicillin-resistant cells.
   - If the transformed cells are spread on agar plates containing ampicillin, only transformants will grow, non transformed recipient cells will die.
6. **Obtaining the Foreign Gene Product**
   - The ultimate aim of recombinant DNA technology is to produce a desirable protein.
   - The foreign gene gets expressed under appropriate conditions.
   - If a protein encoding gene is expressed in a heterologous host, it is called a **recombinant protein**.
   - The cells with foreign genes may be grown on a small scale in the laboratory.
   - The cultures may be used to extract the desired protein and purified using different separation techniques.
   - The cells can also be multiplied on large scale in a continuous culture system.
   - Here, the used medium is drained out from one side while fresh medium is added from the other.
   - It maintains the cells more physiologically active and so produces a larger biomass leading to higher yields of desired protein.

➢ **Bioreactors**
- To produce large quantities of products, the bioreactors are used where large volumes (100-1000 litres) of culture can be processed.

- Bioreactors are the vessels in which raw materials are biologically converted into specific products, enzymes etc. using microbial plant, animal or human cells.
- A bioreactor provides the optimal growth conditions (temperature, pH, substrate, salts, vitamins, oxygen) for achieving the desired product.
- There are two types of bioreactors namely,
  (a) Simple stirred-tank bioreactor
  (b) Sparged stirred-tank bioreactor
- The most commonly used bioreactors are of stirring type.

➢ **Stirred-tank Reactor**

- It is usually cylindrical or with a curved base to facilitate the proper mixing of the reacting contents.
- The stirrer facilitates even mixing and oxygen availability throughout the bioreactor.
- Alternatively, air can be bubbled through the reactor.
- The bioreactor has
  (a) An agitator system.
  (b) An oxygen delivery system.
  (c) A foam control system.
  (d) A temperature control system.
  (e) pH control system.
  (f) Sampling ports (for periodic withdrawal of the culture).
  (g) The contents are mixed by stirrer. This makes the oxygen available throughout the bioreactor.

7. Downstream Processing : All the processes to which the product is subjected to before being marketed as a final and finished product are called as downstream processing.

- It includes a series of processes such as separation and purification of products after the biosynthetic stage.
- The product is formulated with suitable preservatives.
- Such formulation undergoes through clinical trials as in case of drugs.
- Strict quality control testing for each product is also required.
- The downstream processing and quality control testing vary from product to product.

IMPORTANT DIAGRAMS

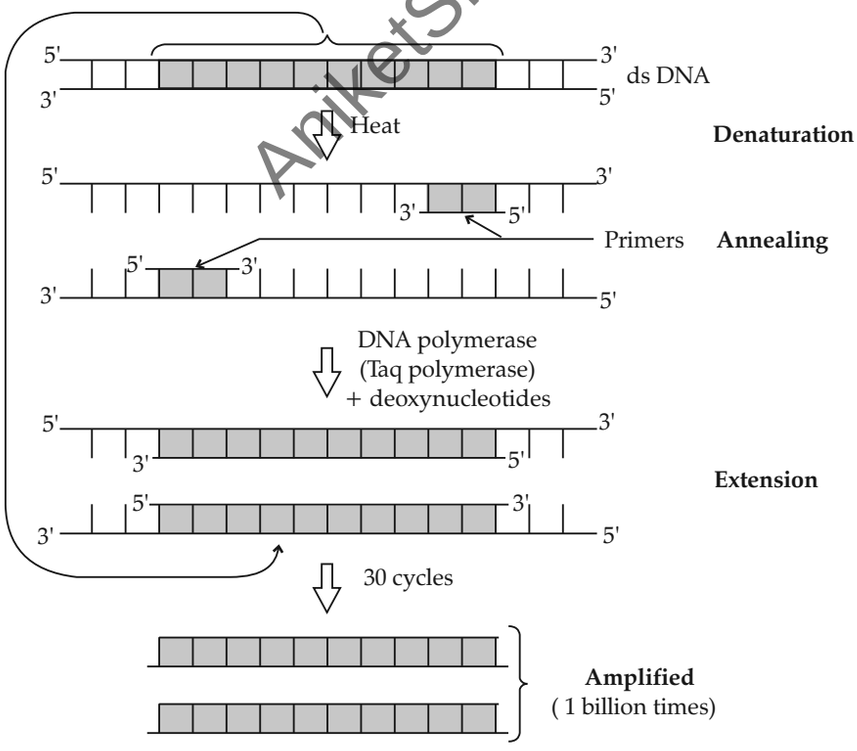


Fig 11.3: Polymerase Chain reaction

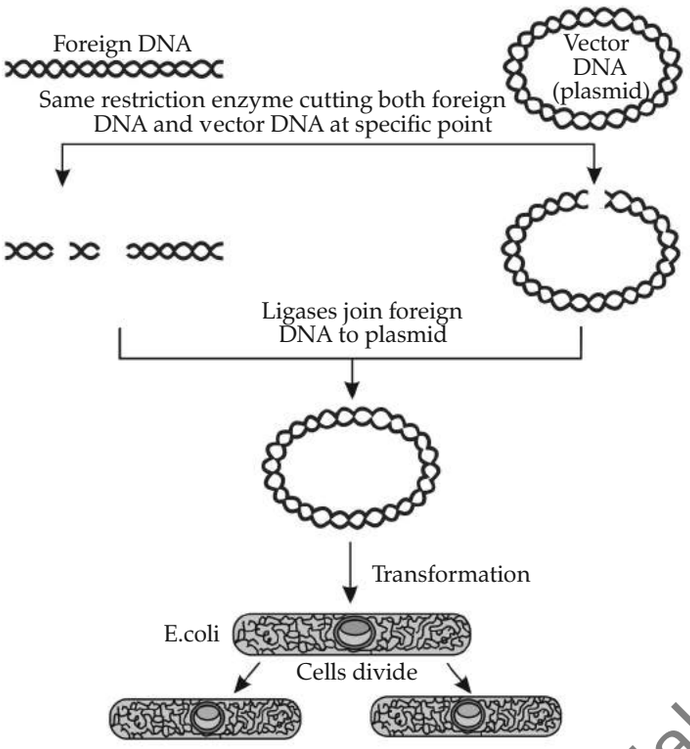


Fig 11.4: Diagrammatic representation of recombinant DNA technology

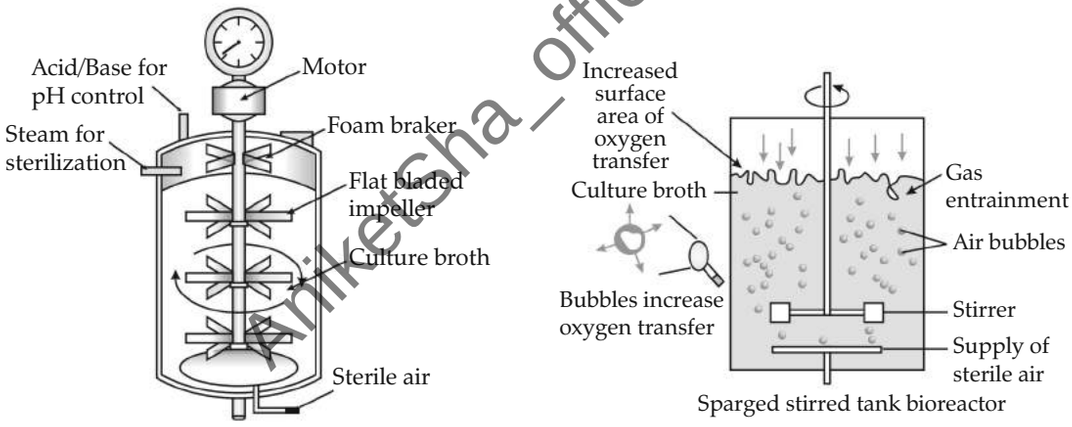


Fig 11.5: Bioreactors- (a) Simple stirred-tank Bioreactor, (b) Sparged stirred-tank bioreactor

Very Short Answer Type Questions (1 mark each)

Q. 1. PCR requires very high temperature conditions where most of the enzymes get denatured. How was this problem resolved in a PCR ?

R **[CBSE SQP 2016-17]**

Ans. The thermostable DNA polymerase is used which is isolated from a bacterium called *Thermus aquaticus*. It remains active during the high temperature this enzyme does not induce denaturation of DNA. This enzymes, taq polymerase carries out amplification of DNA at high temperature. **1**

Q. 2. Write the names of the enzymes that are used for isolation of DNA from bacterial and fungal cells respectively for Recombinant DNA Technology

R **[Delhi Set-II, III, 2014]**

Ans. Lysozyme for bacterial cells, chitinase for fungal cells. ½ + ½

[CBSE Marking Scheme, 2014]

AI **Q. 3. Name the source of the DNA polymerase used in PCR technique. Mention why it is used ?**

R **[Outside Delhi Set-I, II, 2013]**

Ans. *Thermus aquaticus,* is the source of DNA polymerase because it is a heat-stable DNA polymerase. **1**

Q. 4. How can bacterial DNA be released from the bacterial cell for biotechnology experiments ?

R **[Outside Delhi Set-I, 2011]**

Ans. (Breaking the cell open) Treating with lysozyme.

[CBSE Marking Scheme, 2011] 1

Q. 5. Write the function of a bioreactor.

R **[Outside Delhi Comptt. 2017, Set - III]**

Ans. Bioreactors are required to produce large volumes (100 - 1000 litres) of recombinant proteins / desired protein / enzymes. **1**

[CBSE Marking Scheme, 2017]

Short Answer Type Questions-I

(2 marks each)

Q. 1. How is insertional inactivation of an enzyme used as a selectable marker to differentiate recombinants from non-recombinants ?

R **[Delhi Set-I, II, 2014]**

Ans. The presence of chromogenic substrate gives blue coloured colonies, in presence of β-galactosidase. Presence of an insert (recombinant DNA) results into inactivation of the enzyme, colonies with inactivation of β-galactosidase do not produce any colour.

[CBSE Marking Scheme, 2014] 2

Detailed Answer :

The insertion of rDNA into the coding sequence of an enzyme *alpha-galactosidase* leads to the inactivation of the enzyme called insertional inactivation. The recombinants do not produce a blue coloured colonies in the presence of chromogenic substrate while the non-recombinants produce a blue colour. **2**

Answering Tip

- Understand that the gene LacZ is inactivated due to insertion of the foreign DNA, hence recombinant colonies do not express beta-galactosidase, thus, X-Gal (chromogenic substance) cannot be converted into blue- coloured metabolite.

AI **Q. 2. (i) 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.**

(ii) Why is this method of selection referred to as "insertional inactivation" ?

R **[Outside Delhi Set-III, 2012]**

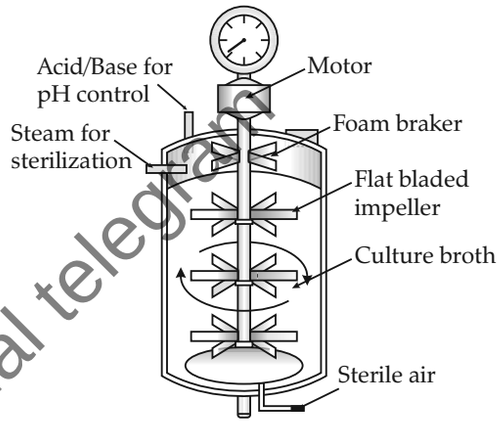
Ans. (i) Bacteria are grown in a medium with chromogenic substrate, colonies formed show blue colour-no recombinants, no blue colour – presence of recombinants.

(ii) Gene for the enzyme is inactivated by insertion.

[CBSE Marking Scheme, 2012] 2

Q. 3. Name the type of bioreactor shown. Write the purpose for which it is used.

R **[Delhi Set-I, 2011]**



Ans. Simple stirred tank bioreactor. Large scale production of recombinant protein / Raw materials are biologically converted into specific products or enzymes, using microbial plants / animals / human cells. **1 + 1 = 2**

[CBSE Marking Scheme, 2011]

Q. 4. Name two commonly used bioreactors. State the importance of using bioreactors.

R **[Delhi Set-I, 2013]**

Ans. The two types of commonly used bioreactors are: (i) Stirred tank bioreactor and **(ii)** Sparged stirred tank bioreactors.

Importance :

A bioreactor also called fermenter is a specialized container or a vessel required for the production of a number of industrial products such as antibiotics, enzymes and vitamins with the help of microorganisms and microbial reactions going on inside the fermenter.

In the bioreactor, a huge amount of raw material or substrates are biologically converted into specific industrial products like vitamins, enzymes, antibiotics, etc. on the industrial scale. **1+1**

Q. 5. What is a primer ? What is its role in PCR ?

R **[Delhi Set-I, II, III, 2011]**

Ans. A primer is a small segment of DNA that binds to a complementary strand of DNA.

Primers are necessary to start the functioning of DNA polymerase enzyme and therefore, are necessary in polymerase chain reaction. **1 + 1**

Q. 6. How can bacterial DNA be released from the bacterial cell for biotechnology experiments ?

R **[Delhi Set-I, II, III, 2011]**

Ans. DNA is enclosed within the membranes; so, we have to break and open the cell to release DNA along with other macromolecules such as RNA, proteins, polysaccharides and also lipids. This can be achieved by treating the bacterial cell / plant or animal tissue with enzymes such as lysozyme (bacteria), cellulase (plant cell), chitinase (fungus). **2**

Q. 7. Why is the 'insertional inactivation' method to detect recombinant DNA preferred to 'antibiotic resistance' procedure? U **[Foreign Set-I, 2016]**

Ans. The presence of a chromogenic substrate gives blue coloured colonies in absence of an insert / in non-transformants, presence of an insert (in the enzyme site), results into (insertional inactivation of the β-galactosidase) colonies which do not produce colour. **½ × 4 = 2**

Antibiotic resistance method requires duplicate plating / cumbersome procedure.

[CBSE Marking Scheme, 2016] 1

Short Answer Type Questions-II **(3 marks each)**

Q. 1. Explain three steps involved in polymerase chain reaction.

R **[Delhi/Outside Delhi, Comptt, Set 1,2,3, 2018]**

OR

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

[Outside Delhi Set-I, 2016]

OR

Any recombinant DNA with a desired gene is required in billion copies for commercial use. How is the amplification done ? Explain.

[Outside Delhi Set-I, II, III, 2010; Delhi Comptt., 2011]

OR

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

[Outside Delhi Set-II, 2012]

Ans. **(i) Denaturation:** Two strands of DNA are separated by heating. **½ × 2**

(ii) Annealing : One set of primers are attached / annealed to the separated DNA (reverse and forward primer) strands. **½ × 2**

(iii) Extension: Taq polymerase catalyses the extension of primers using genomic DNA as template and nucleotides provided in the reaction. **½ × 2**

(correctly labelled diagrams with polarity of strands to be accepted in lieu of explanation)

//

Diagram: Refer Topic 2/ Revision Notes/ Important Diagram/Fig 11.3

[CBSE Marking Scheme, 2018]

Detailed Answer :

Polymerase Chain Reaction Technique can be used to obtain multiple copies of a gene of interest in vitro.

The PCR consists of three steps :

(i) Denaturation : The segment of double stranded DNA of interest is heated to separate the two strands at high temperature of about 90-95°C.

(ii) Annealing : A set of primers (chemically synthesised oligonucleotides which are complementary to the regions of DNA) are annealed to both the separated DNA segments. DNA polymerase (Taq polymerase) extends the primer in the 5′ to 3′ direction.

(iii) Extension : The separated DNA segments acts as templates and primers synthesises new strands along the entire length of DNA strands.

(iv) Amplification : The cycle is repeated several times to generate up to one billion identical copies of DNA.

Commonly Made Error

- While describing PCR, very few candidates mention the exact temperatures which cause Denaturation, Annealing and Extension. Many of the students forget to mention the role of Taq polymerase in this technique.

Answering Tip

- Understand the significance of temperature at each step of PCR technique and the specific reason for using Taq polymerase (temperature resistant).

Q. 2. Why is Taq polymerase preferred to PCR? Mention the source of this enzyme ?

U **[Outside Delhi Set-I, Comptt., 2015]**

Ans. Taq polymerase is used for amplification of DNA / gene, (usually enzymes get denatured). Taq polymerase is thermostable, remains active at high temperature. It is obtained from *Thermus aquaticus.* **1 + 1 + 1 = 3**

[CBSE Marking Scheme, 2015]

Detailed Answer :

Thermostable Taq polymerase extends the primers using nucleotides provided in the reaction and genomic DNA as template.

The source of this enzyme is *Thermus aquaticus.* **2+1**

Commonly Made Error

- Learn to write names of the enzyme- *Thermus aquaticus* with correct spellings according to the rules of binomial nomenclature.

Q. 3. (i) Why was a bacterium used in the first instance of the construction of an artificial recombinant DNA molecule?

(ii) Name the scientists who accomplished this and how? R **[Delhi Set-I, Comptt., 2016]**

Ans. (i) Bacterium has a plasmid in which the desired gene is introduced / the gene to be transferred is from a bacterium. The anti-biotic resistance gene / the host cell (bacterial cell) is required for gene cloning / bacteria produce restriction endonucleases.

(ii) Herbert Boyer and Stanley Cohen

Antibiotic resistant gene was isolated using restriction enzyme and introduced into the plasmid of bacterium *Salmonella typhimurium.* Later the recombinant plasmid was introduced into the bacterium ***E. coli.*** so that it could make copies of gene. **1½ + 1½ = 3**

[CBSE Marking Scheme, 2016]

Q. 4. Write the functions of the following in biotechnology.

(i) Polymerase chain reaction technique

(ii) Restriction endonucleases

(iii) Bacterium *Thermus aquaticus*.

R **[Outside Delhi Set-III, Comptt., 2016]**

Ans. (i) Multiple copies of gene of interest can be obtained.

(ii) They can cut DNA molecule at a particular point by recognizing a specific sequence of base pairs. Thus they are useful in forming recombinant DNA.

(iii) *Thermus aquaticus* is the source of Taq-polymerase which remains active during high temperature induces denaturation of DNA in PCR technique and therefore allows chain reaction to proceed.

[CBSE Marking Scheme, 2016] 3

Q. 5. Draw a labelled sketch of sparged – stirred-tank bioreactor. Write its application.

R **[Delhi Set-I, 2015]**

Ans. Application – Bioreactors are used to produce larger biomass leading to higher yields of desired protein /recombinant protein in bioreactors. Thus, carries the processing of large volume of culture / conversion of raw materials into specific product biologically. **1**

For Diagram: Refer Topic 2/ Revision Notes/ Important Diagrams/ Fig 11.5 (b)

Correct diagram sparged stirred tank bioreactor **1**

Any two correct labellings.

[CBSE Marking Scheme, 2015] ½ + ½

Q. 6 (a) How has the development of bioreactor helped in biotechnology?

(b) Name the most commonly used bioreactor and describe its working?

R **[Delhi/Outside Delhi, 2018]**

OR

Ans. (a) Larger biomass / large volume of culture can be processed leading to higher yields of desired specific products (protein / enzymes), under controlled condition ½ + ½

(b) Stirring type ½

- Mixing of reactor contents evenly (with agitator system or a stirrer). ½
- Facilitates oxygen availability. ½
- Temperature / pH / foam control under optimum conditions. ½

[1 + 2 = 3 marks] [CBSE Marking Scheme, 2018]

AI **Q. 7. (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 enyme in PCR.

R **[Delhi Set-II, III, 2014]**

Ans. (i) (a) Denaturation **(b)** Annealing **(c)** Extension.

(ii) *Thermus aquaticus,* it remains active during the high temperature, (induced to denature double stranded DNA) and catalyses polymerisation of DNA.

[CBSE Marking Scheme, 2014] 3

Q. 8. List the key tools and steps used in recombinant DNA technology. U **[Delhi Set-I, II, III, 2011]**

Ans. Tools are enzymes, vehicle DNA, passenger DNA.

Recombinant DNA technology involves the following steps:

(i) Isolation of DNA.

(ii) Fragmentation of DNA by restriction endo-nucleases.

(iii) Isolation of the desired DNA fragment.

(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.

(vii) Culturing the host cells on a suitable medium on a large scale.

(viii) Extraction of the desired product.

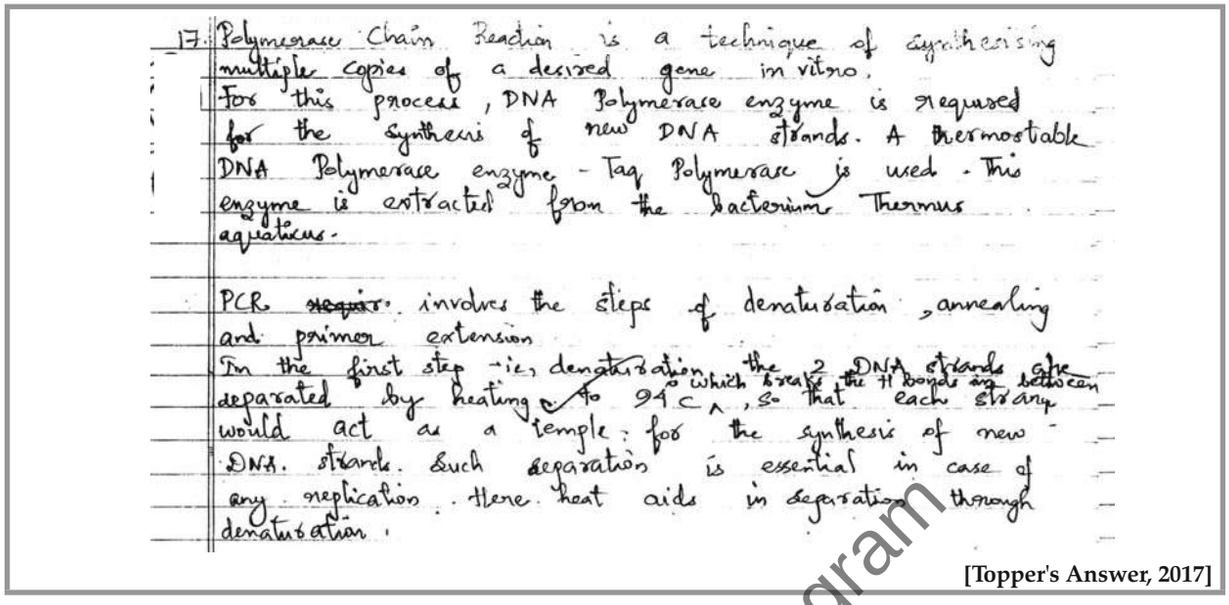
(ix) Downstream processing of the product as a finished product ready for marketing. **3**

Q. 9. Describe the roles of heat, primers and the bacterium *Thermus aquaticus* in the process of PCR. U **[Outside Delhi - 2017, Set - I, II, III]**

Ans. Heat : Denaturation / separation of DNA into two strands. **1**

Primer : Enzyme DNA Polymerase extend the primers using the nucleotides provided in the reaction and the genomic DNA as template. **1**

Thermus aquatics : Source of thermostable DNA polymerase i.e., *Taq polymerase.* **1**



17. Polymerase Chain Reaction is a technique of synthesising multiple copies of a desired gene in vitro. For this process, DNA Polymerase enzyme is requried for the synthesis of new DNA strands. A thermostable DNA Polymerase enzyme - Taq Polymerase is used. This enzyme is extracted from the bacterium Thermus aquaticus.

PCR ~~requir~~ involves the steps of denaturation, annealing and primer extension.
In the first step - ie, denaturation the 2 DNA strands are separated by heating to 94°C, so that each strand would act as a temple for the synthesis of new DNA strands. Such separation is essential in case of any replication. Here heat aids in separation through denaturation. which breaks the H bonds existing between

[Topper's Answer, 2017]

Q. 10. (i) Why is *Taq* polymerase used instead of ordinary DNA polymerase in polymerase chain reaction (PCR) ? Name the source organism of *Taq* polymerase.

(ii) What is PCR used for ?

U **[Delhi Comptt. 2017, Set - III]**

Ans. (i) It is thermostable / remains active during the high temperature induces denaturation of (double stranded) DNA, (bacterium) *Thermus aquaticus.*

(ii) To obtain multiple copies of the gene (or DNA) of interest. **2 + 1**

[CBSE Marking Scheme, 2017]

Detailed Answer :

(i) *Taq* polymerase is used in PCR instead of ordinary DNA because of its thermostable nature. It remains active at high temperature during the denaturation of double stranded DNA. The source organism of *Taq* polymerase is *Thermus aquaticus*.

(ii) Polymerase chain Reaction (PCR) is used to obtain multiple copies of the gene of interest.

AI **Q. 11. How does β-galactosidase coding sequence act as a selectable marker ? Explain. Why it is a preferred selectable marker to antibiotic resistant genes ?** U **[Foreign - 2017, Set - II, III]**

Ans. (i) Presence of a chromogenic substrate gives blue colour, if the plasmid in the bacteria does not have an insert (non-recombinants). ½ + ½

(ii) With the insert-do not produce any colour, recombinant colonies. ½ + ½

(iii) Selection of recombinants due to inactivation of antibiotics, requires simultaneous plating on two plates having different antibiotics / process is more cumbersome. ½ + ½

[CBSE Marking Scheme, 2017]

Q. 12. Write the steps you would suggest to be undertaken to obtain a foreign-gene-product.

R **[Delhi - 2017, Set - I, II, III]**

Ans. Insert a piece of alien or desired or foreign DNA into a cloning vector, transfer it into a Bacterial / plant / animal cell, the alien DNA gets mutiplied, optimised condition (temperature pH, substrate, salts, vitamins, O_2) provided to the culture / culture in bioreactor / in continuous culture system to induce the expression of the target product, extracting the desired product, purifying it by using different separation techniques. ½ × **6**

[CBSE Marking Scheme, 2017]

Detailed Answer :

(i) Insertion of a piece of desired DNA into cloning vector to get recombinant DNA.

(ii) Transfer of recombinant DNA into a host cell. (Plant or animal or bacterial cell).

(iii) The alien DNA will get multiplied.

(iv) After the cloning of gene of interest, optimised conditions are provided to the culture to induce the expression of the target gene.

(v) Extraction of the desired product.

(vi) Purification of desired products by using different separation technique.

Q. 13. Explain three basic steps to be followed during genetic modification of an organism.

R **[Foreign Set- I, II, 2017]**

Ans. (i) Identification of DNA with desirable genes, so that the genetically modified organism has largely desirable genes. ½ + ½

(ii) Introduction of the DNA with desirable genes, into the host using vector. ½ + ½

(iii) Maintenance of introduced DNA in the host and transfer of the DNA to its progeny through cloning. **1**

[CBSE Marking Scheme, 2017]

Q. 14. "A very small sample of tissue or even a drop of blood can help determine paternity". Provide a scientific explanation to substantiate the statement.

C [Outside Delhi, Set-III, 2015]

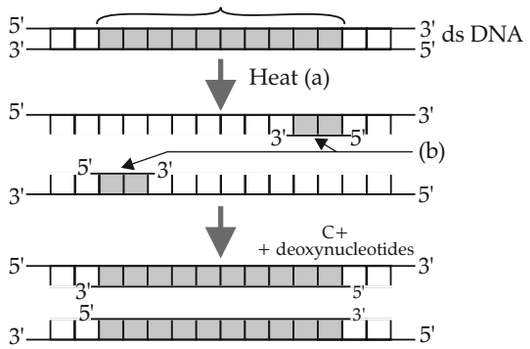
Ans. The above statement can be substantiated through DNA finger printing by southern blot method and by PCR.

DNA from all the cells of an individual shows the same degree of polymorphism.

The polymorphs are heritable. An individual inherits 50% of the chromosomes from maternal and 50% from the paternal parent.

Small amount of DNA from blood or tissue is taken and amplified by PCR, which can be used in DNA finger printing so as to identify the paternity. **3**

Q. 15. A schematic representation of polymerase chain reaction (PCR) upto extension stage is given below.



Answer the questions that follows :

(i) Name the process 'a'

(ii) Identify 'b'

(iii) Identify 'c' and mention its importance in PCR.

A [Foreign 2010]

Ans. (i) Denaturation process.

(ii) Primers.

(iii) Taq DNA polymerase.

Taq polymerase is a thermostable enzyme isolated from a thermophilic bacterium *Thermus aquaticus*. This enzyme remains functional during high temperature required for extension of DNA. **1 × 3 = 3**

Long Answer Type Questions

(5 marks each)

Q. 1. Diagrammatically explain the steps of rDNA technology. [Delhi Set-I, II, III, 2014]

Ans. Diagram: Refer Topic 2/ Revision Notes/ Important diagrams/ Fig 11.4

Q. 2. (i) Describe the different steps in one complete cycle of PCR.

(ii) State the purpose of such an amplified DNA sequence. R [Outside Delhi Set-I, Comptt., 2015]

Ans. (i) Refer SAQ-II/ Q1.
(Same value points to be awarded in an explanation) **½ × 6 = 3**

(ii) Purpose : Used to ligate with a vector for further cloning. Detection of bacteria or virus by amplification of their DNA / detection of HIV in AIDS patient. Detection of mutation in genes in suspected cancer patients. **(Any two) 1+1=2**

AI Q. 3. 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. R [Delhi Set-I, II, III, 2014]

Ans. (i) (a) Identifying the restriction endonuclease that recognises the palindromic nucleotide sequence of the desired gene.

(b) The restriction endonuclease inspects the DNA sequences – finds and recognises the site.

(c) Cuts each of the double helix at the specific point – a little away from the centre of the palindromic site – between the same two bases on the opposite strand.

(d) Makes the over hanging stretch single stranded portion as a sticky end.

(ii) (a) By PCR / Polymerase Chain Reaction.

(b) Desired gene is synthesised in vitro.

(c) DNA is denatured – Annealed using two sets of primers.

(d) Thermostable Taq polymerase extends the primers using nucleotides (provided in the reaction and genomic DNA as template).

(e) Amplified fragments are ligated.

[CBSE Marking Scheme, 2014] 2 + 3 = 5

Know the Terms

- **Genetic engineering :** Techniques to alter the chemistry of genetic material (DNA and RNA), to introduce these into host organisms and thus change the phenotype of the host organism.
- **Restriction enzymes :** Enzymes that are used to cut DNA segment at a specific site are called restriction enzymes.
- **Exonucleases :** Remove nucleotides from the ends of the DNA molecule.

- **Endonucleases :** Make cuts at specific positions within the DNA molecule.
- **Plasmid :** Autonomously replicating circular extra-chromosomal DNA of any bacteria.
- **Origin of replication :** A specific DNA sequence which is responsible for initiating replication is called origin of replication.
- **Vectors :** These are plasmid DNA or viruses that act as vehicle to transfer the piece of DNA attached to it.
- **Palindromic Nucleotide Sequences :** 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.
- **Gel electrophoresis :** A technique which is used to separate the fragments of DNA is known as gel electrophoresis.
- **Transformation :** It is a procedure through which a piece of DNA is introduced in a host bacterium.
- **Insertional inactivation :** The procedure of inserting a recombinant DNA within coding sequence of a functional gene which makes that gene inactive (unable to express) is called insertional inactivation.
- **Selectable Marker :** The gene encoding desirable information useful in identifying and eliminating non-transformants and selectively permitting the growth of the transformants is called selectable marker.
- **Micro-injection :** A technique in which recombinant DNA is directly injected into the nucleus of an animal's cell.
- **Biolistics or Gene gun :** Plant cells are bombarded with high velocity micro-particles of gold or tungsten coated with DNA in a method known as biolistics or gene gun.
- **Bioreactors :** Bioreactors are vessels in which raw materials are biologically converted into specific products, enzymes etc. using microbial plants, animal or human cells.

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