Chapter - 9

Biotechnology: Principles and Processes

Steps involved in Recombinant DNA Technology

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Isolation of DNA from organism by using enzymes like lysozymes (bacterial cells), Chitinase (fungal cell wall), protease (Proteins), RNA s (RNA) and its precipitating in chilled ethanol

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Cutting of DNA at recognition sites by Restriction enzymes. The same enzyme cuts the cloning vector at similar recognition site providing sticky ends.

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The cut fragments are separated using gel-electrophoresis and amplified using PCR.

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The genes (DNA-fragments) are joined with the cloning vector DNA using ligase.

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The re-combinant DNA so formed is transferred into host cell using methods like biolistics, Electroporation, Micro injection or pathogens like bacteria and retroviruses whose pathogenic properties have been removed.

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The host cell containing the r-DNA is cultured in Bioreactors to provide the product at large scale.

↓

The product is separated, purified, (downstream processing) formulated with preservation followed by quality control testing and marketing.

GEL ELECTROPHORESIS

Negatively charged DNA fragments are - Separated by forcing them to move through Agarose gel get aligned towards anode under an electric field.

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The smaller fragments move faster through the gel towards anode

↓

The larger fragments remain near the walls at the cathode end (where poured initially) as they sieve slowly)

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The separated fragments are stained with ethidium bromide and visualized under UV light

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The DNA fragments are cut out from agarose gel by the process known as elution.

↓

These DNA fragments are used in recombinant DNA by joining them with cloning vectors

Polymerase Chain Reaction (PCR)

Denaturation-Separation of DNA into single strand) by applying high temperature upto 95^0C

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Annealing-Two sets of primers (Short stretches of RNA) attach to the single stranded DNA at compementary sites.

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Extension-The primers extend by addition of nucleotides in the presence of thermostable DNA polymerase complimentary to the DNA strand. The primers are removed.

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Repetition- This cycle get repeated so time and the DNA fragments get amplified about one billion times.

Biotechnology : The application of living organisms or of substances made by living organisms to make products for welfare of mankind.

The definition of Biotechnology given by the European Federation of Biotechnology (EFB) : 'The integration of natural science and organisms, cells, parts there of, and molecular analogues for products and services.'

Molecular scissors- Restriction endonuclease
Molecular glues- DNA ligases
Natural genetic engineer- Agrobacterium tumefaciens
Three basic steps involved in creating genetically modified organism (GMO) or transgenic organisms-
(i) Identification of DNA with described genes
(ii) Introduction of the identified DNA into the host
(iii) Maintenance of Introduced DNA into the host and transfer of DNA to its progeny

Principles of Biotechnology :

1. Genetic Engineering : The techniques used to alter the chemistry of genetic material (DNA/RNA) and introduction of it into organisms to change its phenotype.

2. Chemical Engineering : Use of contamination free chemical engineering process of growth of desired microbe or cell in large quantity to obtain bio-technological product like enzyme, antibiotic, vaccine etc.

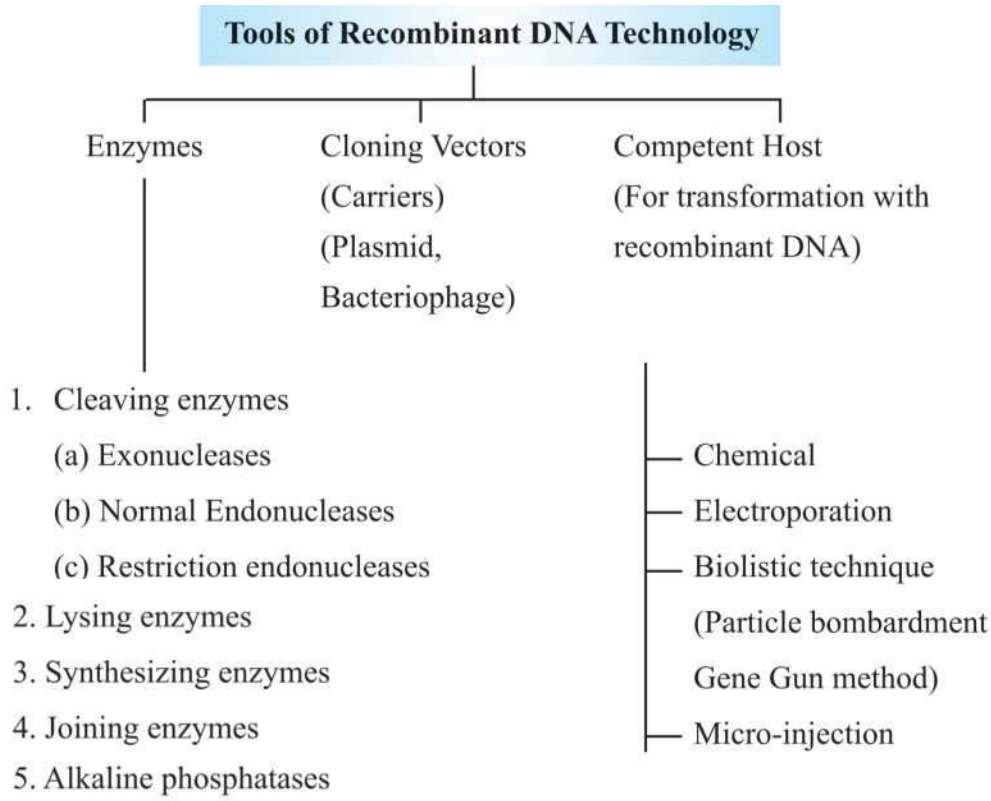
First Artificial reccombinant DNA Molecule :

(i) The two scientists of USA, Stanley Cohen and Herbert Boyer (1972) isolated the antibiotic resistance gene by cutting the desired piece of DNA from the plasmid of the bacterium *Salmonella typhimurium* with the help of restriction enzymes (molecular scissors).

2. This piece of DNA was then linked with the plasmid DNA acting as vector by DNA ligase enzyme.

3. The newly formed recombinant DNA was transferred to bacterium *Escherichia coli* for replication by using the enzyme DNA polymerase. This process is called Cloning.

Recombinant DNA (rDNA) : The hybrid DNA formed by combining DNA segment of two different organisms.



(1) Cleaving Enzymes : These enzymes are used to break DNA molecules.

(a) Exonucleases : Cut off nucleotides from terminal ends of DNA

(b) Endonucleases : Make cut DNA at any point within a DNA.

(c) Restriction Endonucleases : Make cut only specific position within a DNA. Single stranded free ends of DNA which can form hydrogen bonds with their complementary cut DNA segments are called 'Sticky Ends'. These ends can be joined by enzyme ligase.

(2) Lysing Enzymes : These enzymes are used to open the cells to get DNA. For example : Lysozyme is used to dissolve the bacterial cell wall.

(3) Synthesizing :

(a) Reverse Transcriptases : Used in the synthesis of Complementary DNA strands on RNA templates.

(b) DNA Polymerases : Used in the synthesis of Complementary DNA strands on DNA templates.

(4) Joining Enzymes : Are used to join the cut ends of double stranded DNA (act as molecular glue). They join DNA fragments by forming phosphodiester bonds e.g., Ligase.

(5) Alkaline Phosphatases : These enzymes cut the phosphate group from the 5′ end of linearised circular DNA to check its recircularization.

Some Restriction Enzymes

S. No.	Restriction Enzymes	Source	Recognition Site
1.	Alu 1	*Arthrobacter luteus*	↓ 5′-A-G-C-T-3′ 3′-T-C-G-A-5′ ↑
2.	EcoR I	*Escherichia coli* RY 13	↓ 5′-G-A-A-T-T-C-3′ 3′-C-T-T-A-A-G-5′ ↑
3.	Bam H I	*Bacillus amyloliquefaciens*	↓ 5′-G-G-A-T-C-C-3′ 3′-C-C-T-A-G-G-5′ ↑
4.	Sal I	*Streptomyces albus*	↓ 5′-G-T-C-G-A-C-3′ 3′-C-A-G-C-T-G-5′ ↑
5.	Hind II	*Haemophilus influenzae* RD	↓ 5′-G-T-C-G-A-C-3′ 3′-C-A-G-C-T-G-5′ ↑

Palindromic Sequence : Complementary DNA sequences that are the same when each strand is read in the same direction (5′ → 3′). These sequence act as recognition sites for restriction endonuclease.

5′—GAATTC—3′

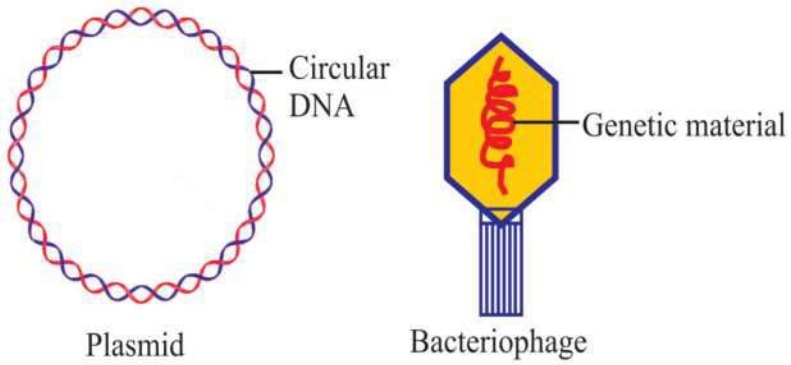
3′—CTTAAG—5′

Complementary DNA (cDNA) : A DNA strand formed from mRNA by using the enzyme reverse transcriptase.

Cloning Vectors : A small, self-replicating DNA molecule into which foreign DNA is inserted. It replicates inside the host cell. The vectors that may be used in genetic engineering are plasmids, bacteriophages, animal, plant, virus, YACs and BACs and some yeasts.

Plasmid : Extra chromosomal, self replicating circular DNA molecule found in certain bacteria and in some yeasts. It has a few genes. Plasmids are used as cloning vectors in genetic engineering. Plasmids were discovered by Willium Haes and Joshua Leduberg in 1952. The most widely used vector in cloning is pBR322. (an artificial plasmid)

Bacteriophage : A virus which infects bacteria is called bacteriophage.



Ti Plasmid : It is an extrachromosomal, double stranded and self replicating DNA molecule found in *Agrobacterium tumifaciens*. It causes tumor in plants. But now Ti Plasmid has been modified into a cloning vector by which desired genes can be delivered into many plants.

Features of cloning vector : Origin of replication (Ori), selectable marker and cloning sites 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 :** It is a gene which helps in identifying and eliminating non-transformants from transformants (having recombinant DNA) by selectively permitting the growth of transformants. The process through which as piece of DNA is introduced in a host bacterium is called transformation. The genes encoding resistance to antibiotics are considered useful selectable marker for *E. coli*.

(c) **Cloning Sites :** A location on a cloning vector into where a foreign gene can be introduced is called recognition site. The vector must have very few (preferably single) recognition sites. The presence of more than one recognition sites within the vector will produce several fragments which will make the process of gene cloning more complicated. Therefore, the foreign DNA is ligated at a restriction site present in one of the two antibiotic resistance gene.

(d) **Small Size of Vector :** This facilitates the intoduction of DNA into the host easily.

Insertional Inactivation : This method is used to differentiate recombinants from non-recombinants on the basis of ability to produce colour in the presence of a chromogenic substrate. When a rDNA is inserted in the coding sequence of an enzyme. It results in inactivation of the enzyme. This is called insertional inactivation.

Case I : The absence of insert in the plasmid of bacteria :

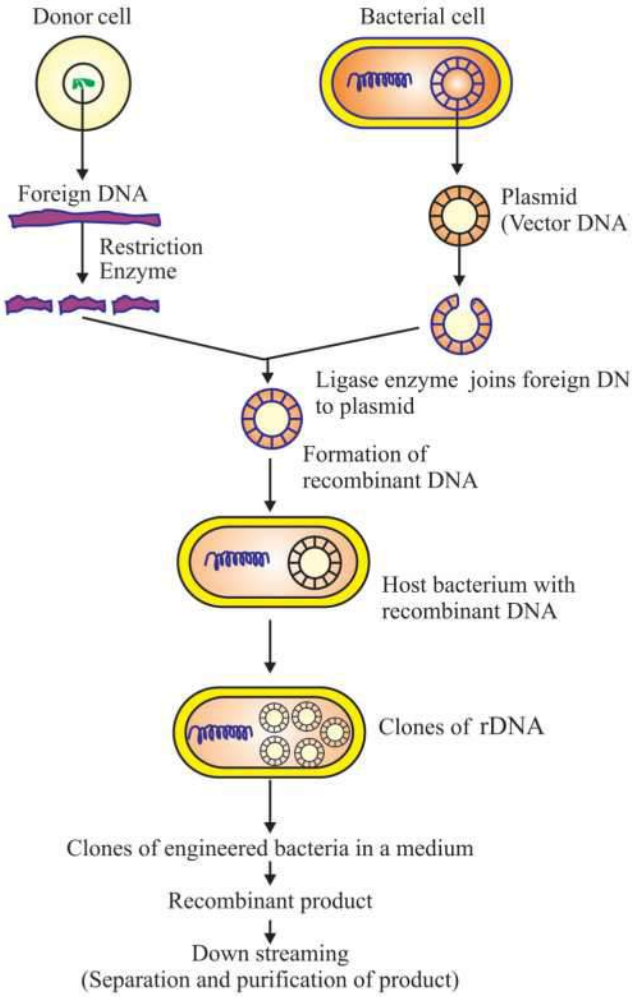
The presence of chromogenic substrate gives blue coloured colonies of bacteria, hence these bacterial colonies are non-recombinant.

Case II : The presence of insert in the plasmid of bacteria :

It results insertional inactivation of the β-gatactosidase, therefore bacterial colonies do not give any colour. Hence the bacterial colonies are recombinant.

Steps in Formation of rDNA by action of EcoRI : EcoRI cuts the DNA between bases G and A only → sticky ends of cut DNAs are formed → DNA fragments join at stickly ends by DNA ligase → Recombinant DNA is formed.

Recombinant DNA Technology :



Process of Recombinant DNA Technology : Isolation of DNA → Cutting of DNA using restriction endonuclease → Amplification of Gene using PCR → Making rDNA and insertion of it into host cell/organism → obtaining the foreign gene product → Downstream processing.

(i) Isolation of Genetic Material (DNA) :

- DNA can be obtained from the cell by treating with enzymes like, Lysozyme for bacteria, Cellulase for plant cell, Chitinase for fungus.
- Histone protein and RNA can be removed by treating with proteases and ribonuclease respectively.
- Purified DNA precipitated by the addition of chilled ethanol, fine threads of DNA are obtained in the suspension.

GEL Electrophoresis :

(1) DNA fragments are separated by forcing them to move towards anode under an electric field through a medium. Agarose gel is used as medium.

(2) Ethidium bromide is used as stain for DNA.

(3) Then on exposure to UV-light appear as orange coloured bands.

(4) Separated bands of DNA are cut out from agrose gel, this is called elution.

(5) These DNA fragments are used in recombinant DNA by joining them with cloning vectors.

(ii) Cutting of DNA at specific location : The purified DNA is cut by use of restriction enzymes. Agarose gel electrophoresis is used to check the progression of restriction enzymes digestion.

(iii) Amplification of gene of interest using PCR : Amplification is the process of making multiple copies of desired DNA segment *invitro*. Polymerase chain reaction involves three steps :

(a) **Denaturation :** The target DNA is heated to high temperature (94°C), resulting the separation of two strands of DNA. Each strand acts as template.

(b) **Annealing :** Two oligonucleotide primers anneal to each of the single stranded DNA template.

(c) **Extension of Primers :** DNA polymerase (*Taq* polymerase) extends the primers using the nucleotides provided in the reactions.

Taq polymerase is a heat stable (Thermostable) DNA polymerase which is isolated from thermophilic bacterium named *Thermus aquaticus*.

(iv) Ligation : The cut out gene of interest from the source of DNA and cut vector with appropriate space, are mixed and ligase enzyme is added. This results recombinant DNA (r-DNA).

(v) Transfer of recombinant DNA into the host : the ligated DNA is introduced into the recipient cell makes itself competent to receive and take up DNA present in the surrounding.

(vi) Obtaining the foreign gene product : The cell containing the foreign gene is cultured on suitable medium and the product can be extracted from the medium.

Bioreactors are used for processing large volume of culture for obtaining products of interest in sufficient quantities. Bioreactor is a large vessel in which raw material is biologically converted into specific product under optimal condition.

(vii) Downstream Processing : The products so obtained undergo a series of processes before putting them in market as a final product. This process includes separation and purification. The products are formulated with suitable preservation and subjected to quality control testing and clinical trials, (in case of drugs).

Questions

VSA **(I Mark)**

1. Write conventional nomenclature of EcoRI.
2. An extra chromosomal segment of circular DNA is used to carry gene of interest into the host cell. What is the name given to it ?
3. Mention the uses of cloning vectors in biotechnology.
4. Identify the recognition sites in the given sequences at which *E.coli* will cut and make sticky ends.

   5′GAATTC-3′
   3′CTTAAG-5′

MULTIPLE CHOICE TYPE QUESTIONS

5. Restriction endonucleose cut

a) Single stranded DNA

b) Single stranded RNA

c) Double stranded DNA

d) Double stranded RNA.

6. Ti phasmid is obtained from

a) Azotobacter

b) Agrobacterium

c) Yeast

d) Rhizobium

7. PCR is required for

a) DNA synthesis

b) DNA amplification

c) Protein synthesis

d) Amino acid synthesis

8. Restriction endo nucleases

a) Break DNA at specific sites

b) Create sticky ends

c) Produce cross - overs

d) Both 'A' and 'B'

9. (I) GAATTC is recognition site for restriction endonuclease.

a) HIND III) b) ECOR I c) Bam I d) Hae III

(ii)Assertion- DNA ligase plays important role in rDNA technology

Reason: Linking of antibiotic resistant gene with plasmid vector became possible by DNA ligase.

a) If both Assertion and Reason are true and Reason is the correct explanation of assertion.

b) If both assertion and reason are true but reason if not the correct explanation of Assertion.

c) If assertion is true but reason is false.

d) If both assertion and reason are false.

10. Assertion : DNA fragments move to words anode in get electrophoresis.

Reason : DNA fragments are negatively charged

a) If both assertion and reason are true and reason is are correct explanation of assertion.

b) If both assertion and reason are true but reason is not the correct explanation of assertion.

c) If assertion is true but reason is false.

d) If both assertion and reason are false.

12. Mr. Sunderam wants to start his business of making drugs at low cost developed by team researcher now, he needs to buy an instrument for his factory. He went to a company to purchase it. The salesman explain features of various types of reactors and Mr. Sunderam purchased the best.

Q.12

I) The optimum conditions in a bio reactor are :

a) Ph, temp, nutrient supply

b) Moisture, Humidity

c) Soil, moisture, Humidity

d) Heat, Cold, Moisture

ii) Downstream processing refers to

a) Making of rDNA

b) Recovery and purification of biosynthetic products

c) Preparation of hybrid DNA

d) Testing of DNA

iii) DNA can be separated in

a) PVC

b) Agar - agar

c) Agoruse gel

d) Hydro gel

iv) The type of bio reactors is

a) Sparged and ring type

b) Sparged and stirred type

c) only sparged type

d) only stirred type

SA- I **(2 Marks)**

13. Name two main steps which are collectively referred to as down streaming process. Why is this process significant ?

14. How does plasmid differ from chromosomal DNA ?

15. (A) bacterial cell is shown in the figure given below. Label the part (A) and (B). Also mention the use of part 'A' in rDNA technology.

16. In the given process of separation and isolation of DNA fragments, some of the steps are missing, Complete the missing steps :

A : Restriction digestion of DNA fragments

↓

B : .....

↓

C : Staining with ethidium bromide

↓

D : Visualisation in U.V. light

↓

E : .....

↓

F : Purification of DNA fragments.

SA-II **(3 Marks)**

17. Since DNA is a hydrophilic molecule, it cannot pass through cell membranes. Name and explain the technique with which the DNA is forced into (i) a bacterial cell (ii) a plant cell (iii) an animal cell.

18. In recombinant DNA technology, vectors are used to transfer a gene of interest in the host cells. Mention any three features of vectors that are most suitable for this purpose.

19. Why is "*Agrobacterium*-mediated genetic engineering transformation" in plants considered as natural genetic engineering ?

20. Observe the given sequence of nitrogenous bases on a DNA fragment and answer the following questions.

5′—CAGAATTCTTA—3′

3′—GTCTTAAGAAT—5′

(a) Name of restriction enzyme which can recognise this DNA sequence.

(b) Write the sequence after digestion.

(c) Why are the ends generated after digestion called sticky ends ?

21. A selectable marker is used in the section of recombinants on the basis of their ability to produce colour in presence of chromogenic substrate.

(a) Mention the name of mechanism involved.

(b) Which enzyme is involved in production of colour ?

(c) How is it advantageous over using antibiotic resistant gene as a selectable market ?

LA **(5 Marks)**

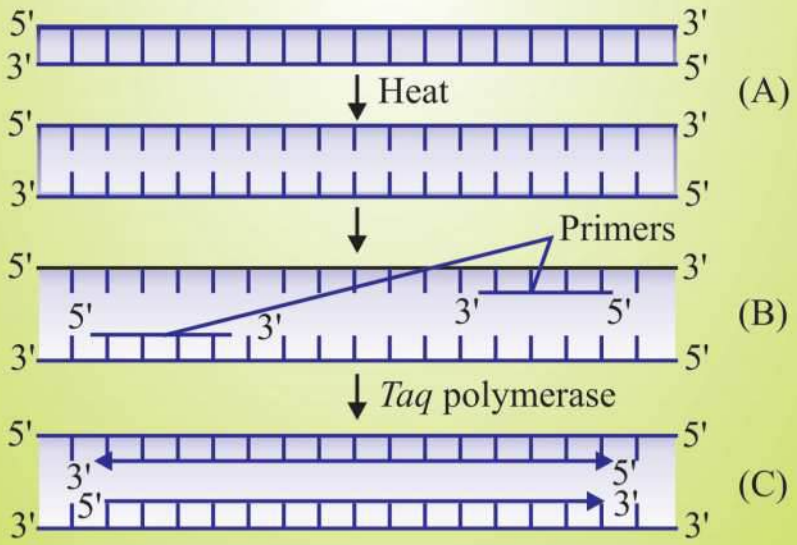
22. The development of bioreactors is required to produced large quantities of products.

(a) Give optimum growth conditions used in bioreactors.

(b) Draw a well labelled diagram of simple stirred-tank bioreactor.

(c) How does a simple stirred tank bioreactor differ from sparged stirred tank bioreactor ?

23. In the given figure, one cycle of polymerase chain reaction (PCR) is shown :

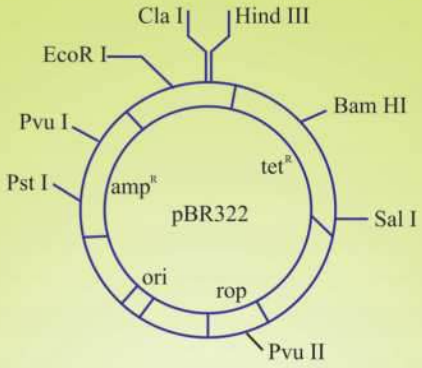


(a) Name the steps A, B and C.

(b) Give the purpose of each of these steps.

(c) State the contribution of *Thermus aquaticus* in this process.

24. Study the figure of vector pBR322 given below in which foreign DNA is ligated at the Bam HI site of tetracyline resistance gene.



Answer the following questions :

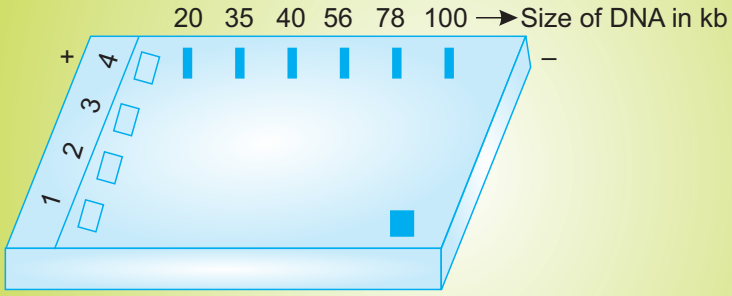
(a) Mention the function of rop.

(b) What will be the selectable marker for this recombinant plasmid and why ?

(c) Explain transformation.

25. A recombinant protein was formed, using the cloning vector pBR322 at the doing side to BamHI. However, the labels test tubes containing, transformants having recombinant and non-recombinant cloning vector got dettached accidentally. How would you differentiate between the two test tubes [Hints: You can use different antibiotics.]

26. While performing get electrophoresis, a student mistakenly attached wrong electrodes i.e., he attached the positive electrode towards the loading the well. Would you expect same results in the process? Why or why not?

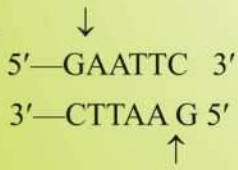
27. 

The above figure shows the result of agrarose gel electrophoresis performed by a student of undigested DNA fragment (lane 1) and digested DNA fragment. However, while drawing the above diagram, student committed a mistake. Identify the mistake and rectify it.

28. Given below is some information of a restriction enzyme:
(i) Isolated form H-strain Bacillus amyloliquefacies
(ii) This was the 3rd restriction enzyme isolated from the said organism.
As per convention, what should be its name?

Answers

VSA (I Mark)

1. E. = *Escherichia* ; co = *coli*; R = Name of Strain; I = order in which enzyme is isolated from strain of bacteria.
2. Plasmid.
3. Gene cloning, gene transfer.
4.


SOLUTION : LESSON 11

MCQ

5. c) 6. b) 7. B) 8. D) 9. B) 10. B) 11. A)

Ans 12.

I) a iii) c

SA-I (2 Marks)

13. • Separation and Purification
    • This process is essential because reaching into market, the product has to be subjected for clinical trial and quality control.

14.

Plasmid DNA	*Chromosomal DNA*
(i) Circular DNA	Linear DNA
(ii) Occurs in bacterial cells	Occurs in nucleus of eukaryotic cells and bacterial cell
(iii) Used as Vector in rDNA technology.	Not used as vector in rDNA technology.

15. (A)—Plasmid, (B)—Nucleoid

Plasmid is used as vector to transfer the gene of interest in the host cell.

8. B—Gel Electrophoresis

E—Elution

SA-II **(3 Marks)**

16. (i) Chemical treatment: treated with divalent cation such as Calcium) and exposure to cold and high temp. (42° C) alternatively (Bacterial cell)

(ii) Biolistics or gene gun. (Plant cell). In this method gold and tungsten particles, coated with DNA are bombarded with high velocity.

(iii) Micro-injection, (animal cell). In this method r DNA is directly injected into the nucleus of an animal cell.

17. (i) Have origin of replication(Ori)

(ii) a selectable marker

(iii) at least one recognition site.

18. *Agrobacterium tumifaciens* is a pathogen in many dicot plants. It is able to deliver a piece of DNA (T-DNA) to transform normal plant cell into a tumor and directs these tumor cells to produce the chemicals required by pathogen.

19. (a) EcoRI

(b)

(c) These are named sticky ends, because they form hydrogen bonds with their complementary cut parts.

20. (a) Insertional inactivation

(b) β-galactosidase.

(c) Selection of recombinants due to inactivation of antibiotics requires simultaneous plating on two plates having different antibiotics.

LA **(5 Marks)**

21. (i) Temperature, pH, substrates, salts, vitamins and oxygen.

(ii) Figure 11.7(a) simple stirred-tank bioreactor Page No. 204 NCERT book, Biology-XII

(iii) The stirrer facilitates even mixing and oxygen availability throughout simple-stirred tank bioreactor, whereas in case of sparged stirred tank bioreactor, air is bubbled throughout the reactor for proper mixing.

22. (A) *Denaturation* : Heat denatures DNA to separate complementary strands.

(B) *Annealing :* Primers hybridises to the denatured DNA strands.

(C) *Thermus aquaticus.* This enzyme induces denaturation of double stranded DNA at high temperature.

(D) *Extension* : Extension of primers resulting in synthesis of copies of target DNA sequence. Enzyme Tag polymerase is isolated from the bacterium.

23. (a) 'Rop' codes for the proteins involved in the replication of plasmid

(b) *Selectable marker* : Ampicillin resistance gene. It will help distinguishing transformants from non-transformants after plating them on ampicillin containing medium.

(c) *Transformation* : It is the phenomenon by which the DNA isolated from one type of cell and introduced into another type, is able to bring about some of the properties of former to the later.

25. The test tube having recombinant DNA transformants will die on adding tetracycline,

26. No, the DNA bands will not be separated due to wrong electrode connection. DNA will not move towards negative electrode.

27. The mistake is that the loading wells have been drawn on the left side instead of right side.

28. Bam HIII