CBSE Class 12 Biology
NCERT Exemplar Solutions
CHAPTER 11
BIOTECHNOLOGY: PRINCIPLES AND PROCESSES

Multiple Choice Questions (MCQs)

1. Rising of dough is due to:

(a) Multiplication of yeast

(b) Production of CO_2

(c) Emulsification

(d) Hydrolysis of wheat flour starch into sugars.

Ans. (b) Production of CO_2

Explanation: Carbon dioxide creates bubbles in the dough and it leads to rising of dough.

2. An enzyme catalysing the removal of nucleotides from the ends of DNA is:

(a) endonuclease

(b) exonuclease

(c) DNA ligase

(d) Hind-II

Ans.(b) exonuclease

Explanation: These are of two kinds; exonucleases and endonucleases. Exonucleases remove nucleotides from the ends of the DNA whereas, endonucleases make cuts at specific positions within the DNA. DNA ligase facilitates joining of sticky ends of DNA fragments. Hind-II is a type of endonuclease.

3. The transfer of genetic material from one bacterium to another through the mediation of a vector like virus is termed as:

(a) Transduction

(b) Conjugation

(c) Transformation

(d) Translation

Ans. (a) Transduction

Explanation: Transfer of genetic materials from a virus or bacterium is called transduction.

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4. Which of the given statement is correct in the context of observing DNA separated by agarose gel electrophoresis?

(a) DNA can be seen in visible light

(b) DNA can be seen without staining in visible light

(c) Ethidium bromide stained DNA can be seen in visible light

(d) Ethidium bromide stained DNA can be seen under exposure to UV light

Ans. (d) Ethidium bromide stained DNA can be seen under exposure to UV light

Explanation: DNA cannot be seen in visible light, so options a and b are incorrect. Staining by ethidium bromide and presence of UV light are necessary to see DNA.

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5. 'Restriction' in Restriction enzyme refers to:

(a) Cleaving of phosphodiester bond in DNA by the enzyme

(b) Cutting of DNA at specific position only

(c) Prevention of the multiplication of bacteriophage in bacteria

(d) All of the above

Ans. (c) Prevention of the multiplication of bacteriophage in bacteria

Explanation: Restriction enzymes belong to a class of enzyme called nuclease. Restriction enzymes prevent the multiplication of bacteriophage in bacteria. They are used for cutting of DNA at specific position which becomes possible by cleaving of phosphodiester bond in DNA by the enzyme. Option 'c' is the correct answer.

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6. Which of the following is not required in the preparation of a recombinant DNA molecules?

(a) Restriction endonuclease

(b) DNA ligase

(c) DNA fragments

(d) E. coli

Ans. (d) E. coli

Explanation: E. coli was used in several experiments involving DNA replication and it has no role in preparation of DNA molecules.

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7. In agarose gel electrophoresis, DNA molecules are separated on the basis of their:

(a) Charge only

(b) Size only

(c) Charge to size ratio

(d) All of the above

Ans. (b) Size only

Explanation: (b) Size only

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8. The most important feature in a plasmid to be used as a vector is:

(a) Origin of replication (ori)

(b) Presence of a selectable marker

(c) Presence of sites for restriction endonuclease

(d) Its size

Ans. (a) Origin of replication (ori)

Explanation: Origin of replication 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. So, if one wants to recover many copies of the target DNA it should be cloned in a vector whose origin support high copy number.

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9. While isolating DNA from bacteria, which of the following enzymes is not used?

(a) Lysozyme

(b)Ribonuclease

(c) Deoxyribonuclease

(d) Protease

Ans. (c) Deoxyribonuclease

Explanation: Lysozyme is used for breaking the membranes which surround DNAs. Ribonuclease is used for removing RNA. Protease is used for removing protein. All these steps are necessary for isolation of DNA from bacteria.

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10. Which of the following has popularised the PCR (polymerase chain reactions)?

(a) Easy availability of DNA template

(b) Availability of synthetic primers

(c) Availability of cheap deoxyribonucleotides

(d) Availability of 'Thermostable' DNA polymerase

Ans. (d) Availability of 'Thermostable' DNA polymerase

Explanation: Thermostable DNA is isolated from a bacterium Thermus aquaticus. It remains active during high temperature induced denaturation of DNA. Before the discovery of thermostable polymerase, the polymerase in use suffered denaturation during heat treatment step of the process.

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11. An antibiotic resistance gene in a vector usually helps in the selection of:

(a) Competent cells

(b) Transformed cells

(c) Recombinant cells

(d) None of the above

Ans. (b) Transformed cells

Explanation: During culture, only transformed cells survive on agar plate. These cells show resistant to antibiotic and can be easily recognized.

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12. Significance of 'heat shock' method in bacterial transformation is to facilitate:

(a) Binding of DNA to the cell wall

(b) Uptake of DNA through membrane transport proteins

(c) Uptake of DNA through transient pores in the bacterial cell wall

(d) Expression of antibiotic resistance gene

Ans. (c) Uptake of DNA through transient pores in the bacterial cell wall

Explanation: Putting the cells with recombinant DNA on ice; followed by a heat shock and then putting them back on ice helps in uptake of DNA through transient pores in the bacterial cell wall.

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13. The role of DNA ligase in the construction of a recombinant DNA molecule is:

(a) Formation of phosphodiester bond between two DNA fragments

(b) Formation of hydrogen bonds between sticky ends of DNA fragments

(c) Ligation of all purine and pyrimidine bases

(d) None of the above

Ans. (a) Formation of phosphodiester bond between two DNA fragments

Explanation: DNA ligase facilitates joining of two sticky ends of DNA fragments by formation of phosphodiester bond between them.

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14. Which of the following is not a source of restriction endonuclease?

(a) Haemophilus influenza

(b) Escherichia coli

(c) Entamoeba coli

(d) Bacillius amyloliquifaciens

Ans. (c) Entamoeba coli

Explanation: Entamoeba coli is not a bacterium and hence is not a source of restriction endonuclease.

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15. Which of the following steps are catalysed by Taq polymerase in a PCR reaction?

(a) Denaturation of template DNA

(b) Annealing of primers to template DNA

(c) Extension of primer end on the template DNA

(d) All of the above

Ans. (c) Extension of primer end on the template DNA

Explanation: (c) Extension of primer end on the template DNA

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16. A bacterial cell was transformed with a recombinant DNA that was generated using a human gene. However, the transformed cells did not produce the desired protein. Reasons could be:

(a) Human gene may have intron which bacteria cannot process

(b) Amino acid codons for humans and bacteria are different

(c) Human protein is formed but degraded by bacteria

(d) All of the above

Ans. (a) Human gene may have intron which bacteria cannot process

Explanation: Any nucleotide sequence in a gene that is removed by RNA splicing during maturation of final RNA product is called intron. Intron can be different in different organisms.

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17. Which of the following should be chosen for best yield if one were to produce a recombinant protein in large amounts?

(a) Laboratory flask of largest capacity

(b) A stirred-tank bioreactor without in-lets and out-lets

(c) A continuous culture system

(d) Any of the above

Ans. (c) A continuous culture system

Explanation: A large flask; without suitable culture system is not going to yield anything. Same holds true for a tank bioreactor. It is the continuous culture system that is a must for producing a recombinant protein.

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18. Who among the following was awarded the Nobel Prize for the development of PCR technique?

(a) Herbert Boyer

(b) Hargovind Khurana

(c) Kary Mullis

(d) Arthur Kornberg

Ans. (c) Kary Mullis

Explanation: (c) Kary Mullis

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19. Which of the following statements does not hold true for restriction enzyme?

(a) It recognises a palindromic nucleotide sequence

(b) It is an endonuclease

(c) It is isolated from viruses

(d) It produces the same kind of sticky ends in different DNA molecules

Ans. (c) It is isolated from viruses

Explanation: It is isolated from bacteria.

CBSE Class 12 Biology
NCERT Exemplar Solutions
CHAPTER 11
BIOTECHNOLOGY: PRINCIPLES AND PROCESSES

Very Short Answer Types Questions

1. How is copy number of the plasmid vector related to yield of recombinant protein?

Ans. A higher number of copies of plasmid vector helps in producing a large quantity of recombinant protein.

2. Would you choose an exonuclease while producing a recombinant DNA molecule?

Ans. Exonuclease removes nucleotides from the ends of the DNA and hence it cannot help in producing circular DNA. So, exonuclease cannot be used for making a recombinant DNA molecule.

3. What does H in' 'd' and 'III' refer to in the enzyme Hind III?

Ans. In the enzyme Hind III; 'H in' refers to Haemophilus influenza, D refers to the strain of H. influenza and III refers to the sequence in which this enzyme was discovered.

4. Restriction enzymes should not have more than one site of action in the cloning site of a vector. Comment.

Ans. Presence of more than one recognition site on vector will generate many fragment of DNA; which will complicate the matter. Hence, restriction enzymes should have, one recognition site at the vector.

5. What does 'competent' refer to in competent cells used in transformation experiments?

Ans. The ability of the bacterial cell to take up DNA through pores in cell wall is called

competence. DNA is a hydrophilic molecule and hence, it cannot pass through the cell membrane. So, cell is made 'competent' by treating with suitable divalent ion; like calcium.

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

Ans. Protease helps in removing protein during the process of obtaining pure DNA. Other macromolecules are eliminated with the help of suitable enzymes during this process. At the end, pure DNA is isolated.

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

Ans. If denaturation is missed then primers will not be able to join at the template. This will result in no extension, no amplification and thus a large number of copies of DNA cannot be made.

8. Name a recombinant vaccine that is currently being used in vaccination program.

Ans. Hepatitis B vaccine

9. Do biomolecules (DNA, protein) exhibit biological activity in anhydrous conditions?

Ans. Biomolecules do not exhibit biological activity in anhydrous conditions. DNA may get damaged under anhydrous condition but has the ability to repair later on. Protein molecule may get denatured under anhydrous conditions.

10. What modification is done on the Ti plasmid of Agrobacterium tumefaciens to convert it into a cloning vector?

Ans. Ti plasmid in Agrobacterium has the ability to induce tumor in plants. This plasmid is 'disarmed' by suitable modification and then it can be used as a cloning vector for delivering gene of interest to plants and animals.

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NCERT Exemplar Solutions
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Short Answer Type Questions

1. What is meant by gene cloning?

Ans. A set of experimental methods used to assemble recombinant DNA molecules and to use them for cloning in host organism is called gene cloning. Gene cloning involves following main steps:

- Selection of a suitable gene.
- Treating the gene to obtain small fragments.
- Transferring the fragment in a suitable host.

2. Both a wine maker and a molecular biologist who had developed a recombinant vaccine claim to be biotechnologists. Who in your opinion is correct?

Ans. The basic definition of biotechnology says is as a set of methods to use live organisms to produce products and processes for the benefit of human kind. So, it is correct to include a wine maker as well as a molecular biologist under the category of biotechnologist. They have developed a recombinant vaccine which will definitely increase the production of vaccine for human welfare.

3. A recombinant DNA molecule was created by ligating a gene to a plasmid vector. By mistake, an exonuclease was added to the tube containing the recombinant DNA. How does this affect the next step in the experiment i.e. bacterial transformation?

Ans. When a DNA molecule is created by ligated a gene to a plasmid vector; it becomes a circular DNA which is ready to replicate in host organism. After this stage, addition of exonuclease is not going to affect the process because the DNA does not have a free end and hence enzyme exonuclease will not get a substrate to show its action. So, in this experiment;

bacterial transformation is not going to be disturbed.

4. Restriction enzymes that are used in the construction of recombinant DNA are endonucleases which cut the DNA at 'specific-recognition sequence'. What would be the disadvantage if they do not cut the DNA at specific-recognition sequence?

Ans. Specific-recognition sequence in a DNA provide sticky ends at which recombination of genes takes place. This further leads to replication of selected gene. If endonuclease fails to cut DNA at specific-recognition sequence; then recombination or replication will fail to take place.

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

Ans. A circular DNA opens up to resemble a single linear DNA. A Linear DNA is divided into two fragments after cleavage. Hence, circular DNA shows one band, while linear DNA shows two bands.

6. How does one visualise DNA on an agarose gel?

Ans. DNA fragments separate when they are moved towards anode in and electric field. Agarose gel provides the matrix through which DNA fragments separate due to sieving effect. Separated DNA fragments can be visualized only after staining with ethidium bromide and then by exposure to UV radiation. After staining, DNA fragments appear as bright orange bands.

7. A plasmid without a selectable marker was chosen as vector for cloning a gene. How does this affect the experiment?

Ans. Selectable marker helps in identifying and eliminating non-transformant DNA and in selectively permitting the growth of transformants. In the absence of a selectable marker, it will not be possible to differentiate between transformants and non-transformants. Thus, carrying the experiment to its logical end would be impossible in the absence of selectable

marker.

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

Ans. Following are the possible reasons for non-observation of DNA bands:

(a) DNA may have got contaminated because of accidental additional of nuclease enzyme.

(b) Electrodes may have been put in wrong orientation; with anode towards the loading well. As DNA is negatively charged, it moves towards anode. When anode is near the loading well, separated DNA may move out of the gel.

(c) Quantity of ethidium bromide may not have been sufficient.

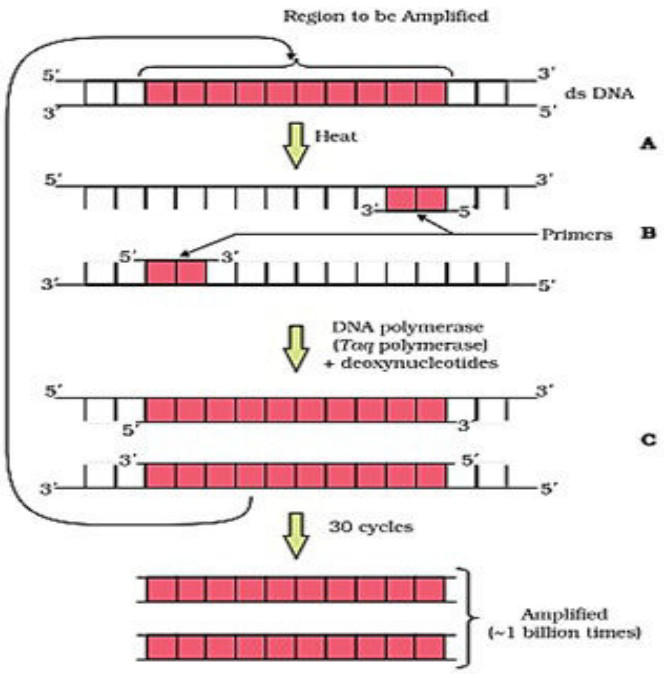
9. Describe the role $CaCl_2$ of in the preparation of competent cells?

Ans. A divalent ions increases the efficiency of uptake of DNA through the pores in bacterial cell wall. $CaCl_2$ provides the divalent ion Ca^{2+} which create transient pores on the bacterial cell wall which facilitate entry of foreign DNA into the bacterial cells. Loading of a divalent ions make the cell competent.

10. What would happen when one grows a recombinant bacterium in a bioreactor but forget to add antibiotic to the medium in which the recombinant is growing?

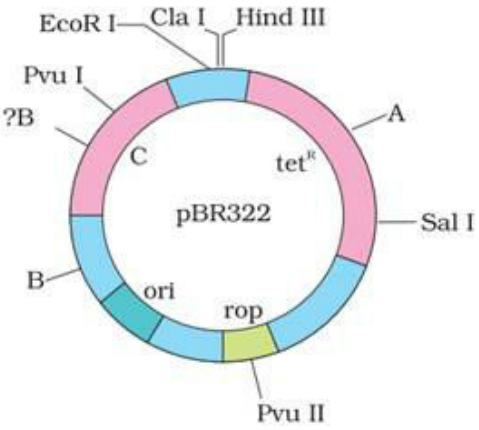
Ans. In the absence of antibiotic, the recombinant bacterium does not need to produce a gene which can make it resistant to the antibiotic. In other words, there is no pressure on the recombinant bacterium to make the desirable gene. Thus, in the absence of antibiotic; a gene of interest will not be produced by the recombinant bacterium.

11. Identify and explain steps ‘A’, ‘B’ and ‘C’ in the PCR diagram given below.

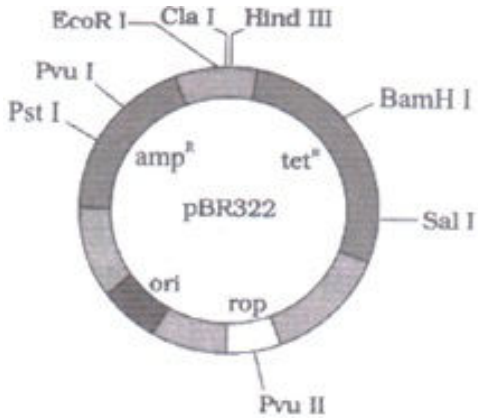


Ans. A: Denaturation, B: Annealing, C: Extension

12. Name the regions marked A, B and C.



Ans.



- A shows tetracycline resistance site
- B shows restriction site Pst I
- C shows ampicillin resistance site

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Long Answer Type Questions

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

Ans. A marker gene helps in differentiating between transformant genes and non-transformant genes. This helps in selecting the suitable recombinants. In case of E. coli; pBR322 is the vector for resistance to antibiotic tetracycline. The insertional inactivation of pBR322 will result in loss of resistance to tetracycline by E. coli. This can be found out by growing the recombinants on two plates; one containing tetracycline and another containing ampicillin. The recombinant will grow in ampicillin but not in tetracycline. A marker gene for chromogenic substrate helps in identifying the recombinant DNA on the basis of gain or loss of colour from the chromogenic substrate. Insertional inactivation of a marker gene coding for a chromogenic substrate will result in no blue colour imparted in the colony.

These steps are taken for easy identification of recombinants from non-recombinants.

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2. Describe the role of Agrobacterium tumefaciens in transforming a plant cell.

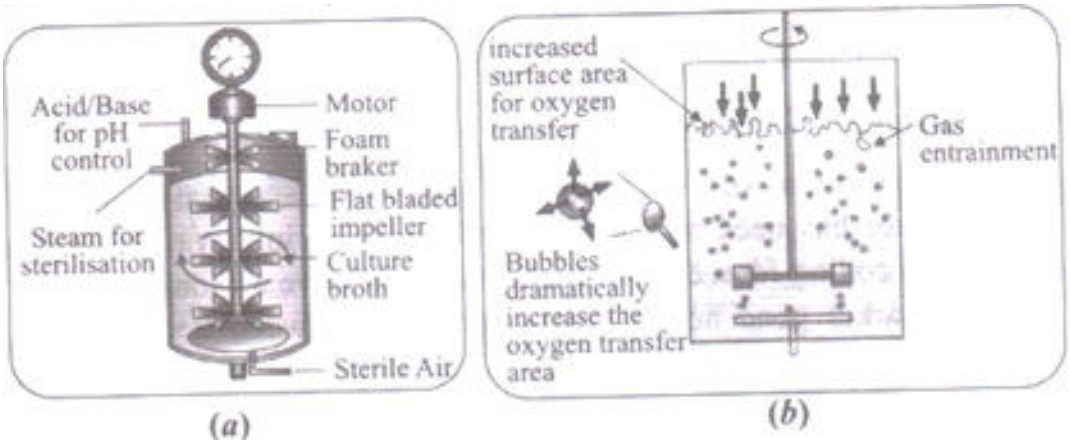
Ans. Agrobacterium tumefaciens infects walnuts, grapevines, sugar beets, horse radish, etc.

- Agrobacterium tumefaciens infects plants usually through an open wound.
- Once it enters its plant host, it injects a section of its DNA called the T-DNA which is derived from its Ti (tumor inducing) plasmid into its host.
- The T-DNA first directs the plant cells to make auxins and cytokinins, which causes the cells to become irregularly shaped and form a visible tumor called a gall.
- The T-DNA then directs the plant cell to start making opines (usually nopaline or agropine) which A. tumefaciens use as an energy source. Thus, A. tumefaciens creates

a special niche for itself inside the gall.

Thus, this property of Ti-plasmid has been exploited for cloning of gene of interest and stably integrating them in the plant genes. Therefore, by using Ti-plasmid or its derivations, recombinant plant cells with desired genes of interest stably integrated in the plant genome has been successfully produced.

3. Illustrate the design of a bioreactor. Highlight the difference between a flask in your laboratory and a bioreactor which allows cells to grow in a continuous culture system.



(a) Simple stirred-tank bioreactor;

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

Structure of Bioreactor:

- It is a cylindrical structure with a curved base.
- A stirrer is present for even mixing and oxygen availability throughout the reactor.
- There is an agitator system, an oxygen delivery system, a foam control system, a temperature control system, etc.
- There is a sampling port through which small volumes of culture can be taken out periodically.

A flask in a laboratory cannot be used for producing recombinant DNA on large scale. Unlike a bioreactor; a flask can not be used to grow culture continuously.