

MULTIPLE-CHOICE QUESTIONS

1. Rising of dough is due to:

- a. Multiplication of yeast**
- b. Production of CO₂**
- c. Emulsification**
- d. Hydrolysis of wheat flour starch into sugars.**

Solution:

Option (b) is the answer.

2. Which of the following enzymes catalyse the removal of nucleotides from the ends of DNA?

- a. endonuclease**
- b. exonuclease**
- c. DNA ligase**
- d. Hind – II**

Solution:

Option (b) is the answer.

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

- a. Transduction**
- b. Conjugation**
- c. Transformation**
- d. Translation**

Solution:

Option (a) is the answer.

4. Which of the given statements is correct in the context of visualizing DNA molecules 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**

Solution:

Option (d) is the answer.

5. 'Restriction' in Restriction enzyme refers to:

- a. Cleaving of the phosphodiester bond in DNA by the enzyme**
- b. Cutting of DNA at a specific position only**
- c. Prevention of the multiplication of bacteriophage by the host bacteria**
- d. All of the above**

Solution:

Option (c) is the answer.

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

- a. Restriction endonuclease**
- b. DNA ligase**
- c. DNA fragments**
- d. E.coli**

Solution:

Option (d) is the answer.

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

- a. Charge the only**
- b. Size only**
- c. Charge to size ratio**
- d. All of the above**

Solution:

Option (d) is the answer.

8. The most important feature in a plasmid to serve as a vector in gene cloning experiment is:

- a. Origin of replication (ori)**
- b. Presence of a selectable marker**
- c. Presence of sites for restriction endonuclease**
- d. Its size**

Solution:

Option (a) is the answer.

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

- a. Lysozyme**
- b. Ribonuclease**
- c. Deoxyribonuclease**
- d. Protease**

Solution:

Option (c) is the answer.

10. Which of the following contributed in popularising the PCR (polymerase chain reactions) technique?

- a. Easy availability of DNA template**
- b. Availability of synthetic primers**
- c. Availability of cheap deoxyribonucleotides**
- d. Availability of 'Thermostable' DNA polymerase**

Solution:

Option (d) is the answer.

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

- a. Competent bacterial cells
- b. Transformed bacterial cells
- c. Recombinant bacterial cells
- d. None of the above

Solution:

Option (b) is the answer

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

Solution:

Option (c) is the answer.

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

- a. Formation of a 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

Solution:

Option (a) is the answer.

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

- a. *Haemophilus influenzae*
- b. *Escherichia coli*
- c. *Entamoeba coli*
- d. *Bacillus amyloliquefaciens*

Solution:

Option (c) is the answer.

15. Which of the following steps are catalysed by Taq DNA 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

Solution:

Option (c) is the answer.

16. A bacterial cell was transformed with a recombinant DNA molecule that was generated using a human gene. However, the transformed cells did

not produce the desired protein. Reasons could be:

- a. 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

Solution:

Option (a) is the answer.

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

Solution:

Option (c) is the answer.

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

Solution:

Option (c) is the answer.

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

- a. It recognises a palindromic nucleotide sequence
- b. It is an endonuclease
- c. It is isolated from viruses
- d. It can produce the same kind of sticky ends in different DNA molecules

Solution:

Option (c) is the answer.

VERY SHORT ANSWER TYPE QUESTIONS

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

Solution:

Copy number decides the times to which the rDNA can be multiplied. More is the copy number higher amount of the recombinant protein is produced.

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

Solution:

No, exonuclease removes nucleotide from the end of the DNA strands.

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

Solution:

H refers to the genus of the organism from which enzyme has been isolated. H refers to Haemophilus. d refers to the strain type. III refers to the sequence in which the restriction enzyme is isolated.

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

Comment.

Solution:

It would result in the formation of multiple fragments if a restriction enzyme would have more than one site of action.

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

Solution:

Competent cell refers to the capability of the cell wall of bacteria to uptake hydrophilic DNA fragments when treated with divalent ions through the cell membrane

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

Solution:

Proteases are the enzymes which digest the protein present inside the cell. Proteases ensure the isolation of DNA without its contamination with proteins. Protein causes interference during downstream processing.

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

Solution:

If the denaturation step is missed, DNA strands would not separate from each other hence primer could not be added to the ends.

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

Solution:

The hepatitis-B vaccine is the recombinant vaccine that is currently being used in a vaccination program

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

Solution:

No. In the absence of water, biomolecules become dysfunctional.

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

Solution:

Ti plasmid of Agrobacterium tumefaciens possesses the capacity to induce tumour formation.

SHORT ANSWER TYPE QUESTIONS

1. What is meant by gene cloning?

Solution:

Gene cloning involves the insertion of a gene of interest within the DNA of a vector. These vectors are

used to introduce the gene of interest inside the host cell.

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

Solution:

Both are correct. The winemaker uses yeast to produce ethanol (natural phenomenon) whereas, a molecular Biologist uses vector with high copy number to produce a large number of antigens.

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?

Solution:

It would not affect the constructed recombinant DNA. The endonuclease would not recognise the specific palindromic nucleotide sequences and will not cut at specific positions as the recombinant DNA has no free ends.

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?

Solution:

If restriction enzymes don't cut the DNA at specific positions, it would not produce sticky ends and construction of recombinant DNA would not be possible.

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.

Solution:

When endonuclease cuts the specific restriction site of the circular DNA of plasmid, the circular DNA changes into linear DNA. But, when endonuclease cuts within the linear DNA it would result in the formation of two linear DNA fragments.

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

Solution:

DNA is made visible by staining it with Ethidium bromide, which on exposure with UV rays appears to be orange coloured bands.

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

Solution:

In the absence of a selectable marker, it would become difficult to distinguish between transformant and non-transformant cell.

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?

Solution:

Maybe the isolated DNA has been degraded during the isolation process or DNA has not been exposed

to UV radiations after staining it with Ethidium bromide.

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

Solution:

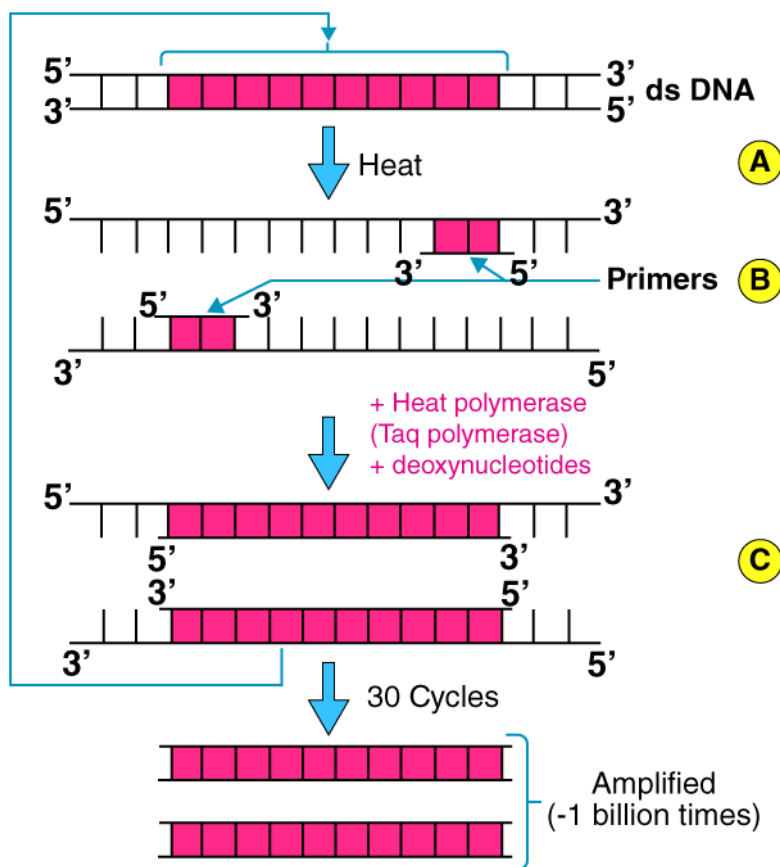
When Ca^{2+} ions are rubbed against the cell wall it results in enlargement of the diameter of the pores and allows the uptake of foreign DNA fragment.

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?

Solution:

In the absence of an antibiotic mixture of both transformants and non-transformants will be produced. It would result in the production of poor quality yield.

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



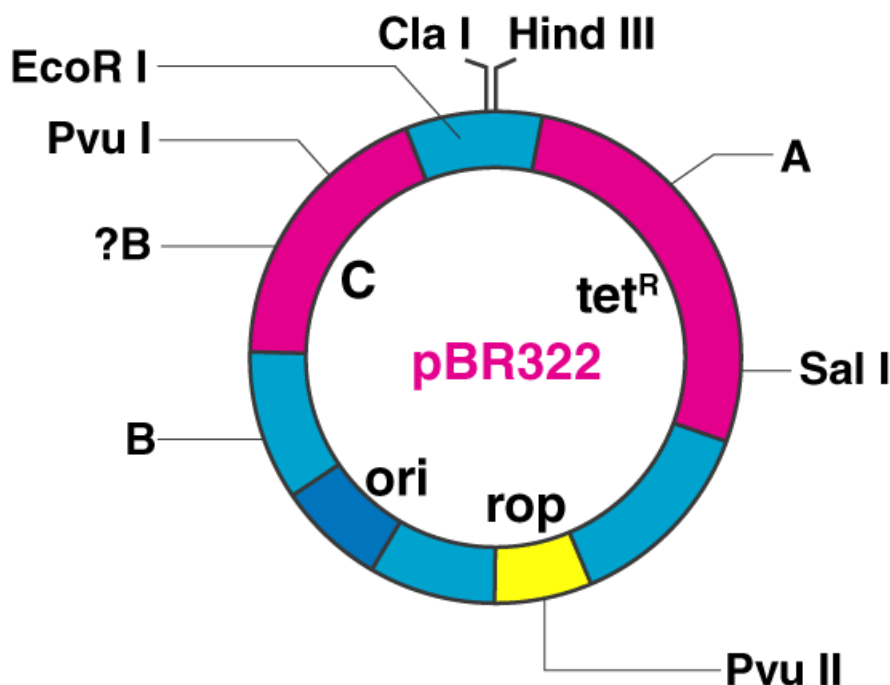
Solution:

A. Denaturation- in this step double-stranded DNA is denatured at a high temperature of 94°C for 15 seconds.

B. Annealing-Primers are added to anneal. This is done at 450 C using Mg^{2+} ions.

C. Extension- Taq polymerase is used to extend the primer by adding nucleotides at a temperature of 72°C.

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



Solution:

A- Bam HI

B- Pst I

C- amp^R

LONG ANSWER TYPE QUESTIONS

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

Solution:

Insertional inactivation of antibiotic markers requires simultaneous plating of two culture media or chromogenic inactivation is a short method to distinguish between transformants and non-transformants. In chromogenic inactivation, the gene of interest is inserted in between the chromogenic gene. Due to the inactivation of the chromogenic gene, the recombinant DNA would not produce colour. Hence it can be concluded that Colonies producing colour are identified as non-transformants whereas the colonies which do not produce colour are transformant colonies.

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

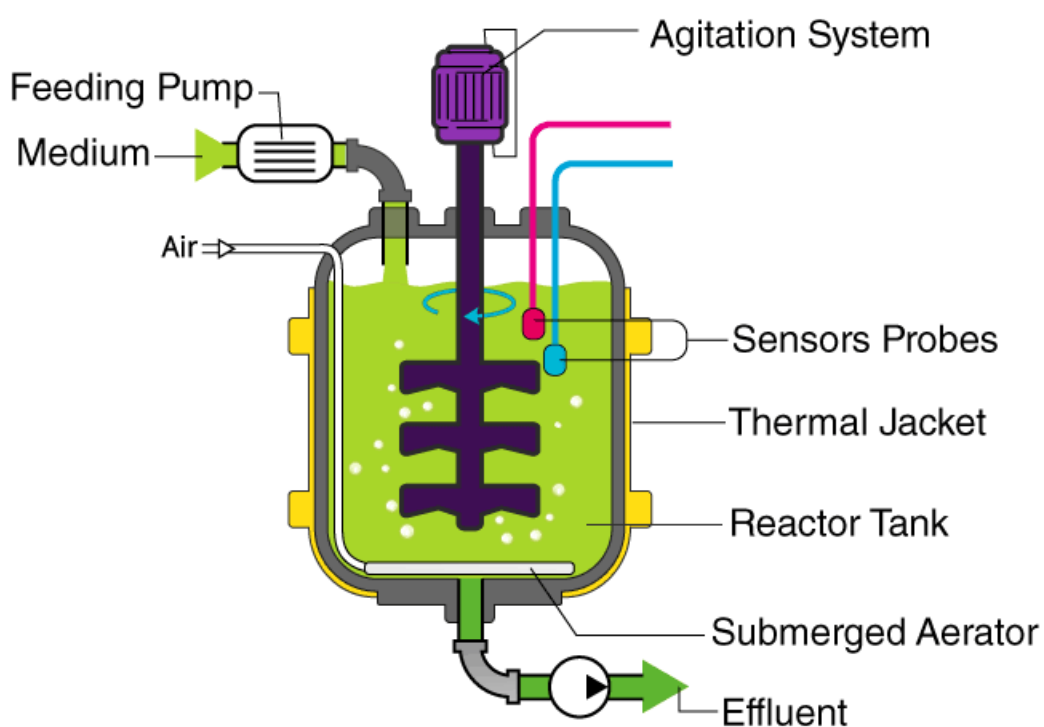
Solution:

Agrobacterium tumefaciens is known to cause infection in plants especially in dicots.

The Ti plasmid of this bacterium induces tumour formation inside the plant cell and has been exploited for cloning of the gene of interest and stably integrating them in the plant genes. The DNA of Ti plasmid gets incorporated with the host cell DNA. However, to use it as a vector the tumour causing gene has been deleted by a molecular biologist.

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.

Solution:



Flask is used to small laboratory scale testing of culture and bioreactor is used for commercial-scale production.

In a flask, the small volume cultures cannot yield appreciable quantities of products whereas in bioreactor the large quantities can be cultured that is about volume 100-1000litres.