

# Biotechnology Principles and Processes - Exemplar Solutions

## Multiple Choice Questions

**1. Rising of dough is due to**

- (a) multiplication of yeast
- (b) production of CO<sub>2</sub>
- (c) emulsification
- (d) hydrolysis of wheat flour starch into sugars

**Ans:** (b) production of CO<sub>2</sub>

**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) Exonucleases

**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

**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.

**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:** (b) cutting of DNA at specific position only

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

- (a) Restriction endonucleases

- (b) DNA ligase
- (c) DNA fragments
- (d) *E. coli*

Ans: (d) *E. coli*

**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 these

Ans: (b) size only

**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)

**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

**10. Which of the following has popularised the PCR (Polymerase Chain Reaction)?**

- (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

**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 these

Ans: (b) transformed cells

**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

**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

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

- (a) *Haemophilus influenzae*
- (b) *Escherichia coli*
- (c) *Agrobacterium tumefaciens*
- (d) *Bacillus amyloli*

Ans: (c) *Agrobacterium tumefaciens*

**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

**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

**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 inlets and outlets
- (c) A continuous culture system
- (d) Any of the above

Ans: (c) A continuous culture system

**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

**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

### Very Short Answer Type Questions

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

Ans: The recombinant DNA can multiply as many times as the copy number of the vector plasmid, thereby determining the yield of recombinant protein. So, the higher the copy number of the plasmid vector, the higher the copy number of the gene and consequently, the protein coded by the gene is produced in high amounts.

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

Ans: No, as exonuclease acts on the free ends of a linear DNA molecule. Therefore, instead of producing DNA fragments with sticky ends, it will shorten or completely degrade the DNA fragment containing the gene of interest, and the circular plasmid (vector) will not be cut as it lacks free ends.

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

Ans:

- (i) The first letter 'H' indicates the genus of the organism from which the enzyme was isolated, H = genus Haemophilus.
- (ii) The fourth letter d indicates the particular strain used to produce the enzyme, d = strain Rd.
- (iii) The Roman numerals denoted the sequence in which the restriction endonuclease enzyme from that particular genus, species and strain of bacteria has been isolated-III, i.e., the third restriction endonuclease to be isolated from this species.

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

**Comment.**

Ans: If the restriction enzymes have more than one recognition site in a vector, then the vector itself will be fragmented on treatment with the restriction enzymes.

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

Ans: DNA, being a hydrophilic molecule, can not pass through cell membranes. Therefore, the bacteria should be made competent to accept the DNA molecules. Competent means bacterial cells, on treatment with chemicals like  $\text{CaCl}_2$ , are made capable of taking up foreign DNA.

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

**Ans:** Proteases degrade the proteins present inside a cell (from which DNA is being isolated). If the proteins are not removed from the DNA preparation, then they could interfere with any downstream treatment of the DNA.

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

**Ans:** If denaturation of double-stranded DNA does not take place, then primers will not be able to anneal (join) to the template. Hence, no extension will take place and after are there will be no amplification.

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

**Ans:** Hepatitis-B recombinant vaccine (Engerix) is used for vaccination against hepatitis B.

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

**Ans:** Biomolecules (DNA and protein) exhibit a change in biological activity in anhydrous conditions. In non-aqueous or anhydrous conditions, the rigidity of protein and DNA increases due to the weakening of hydrogen bond strength.

It results in a change in overall free energy, which is the combined effect of the exposure of the interior polar and non-polar groups and their interaction with water.

In the absence of aqueous conditions, the free energy change is negative, which is responsible for the denaturation of biomolecules.

The increasing strength of the hydrogen bond causes water to primarily bond with itself and not be available for the hydrating structure of proteins or DNA, or for dissolving ions.

On the other hand, if the water-water hydrogen bond strength reduces, then the exchange mechanisms operating within the cell, such as hydrogen-bonded water chains within and between proteins and DNA, will become non-operational. It will further lead to the denaturation.

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

**Ans:** The plasmid is disarmed by deleting the tumour-inducing genes in the plasmid. So, it becomes an effective cloning vector. The modified tumour-inducing (Ti) plasmid of Agrobacterium tumefaciens will no longer remain pathogenic to the plants but still deliver genes of interest into a variety of plants.

## Short Answer Type Questions

**1. What is meant by gene cloning?**

**Ans:** Gene cloning refers to a process in which a gene of interest is ligated to a vector. The recombinant DNA thus produced is introduced into a host cell by transformation. Each cell gets one DNA molecule, and when the transformed cell grows to a bacterial colony, each cell in the colony has a copy of the gene. This is gene cloning.

**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:** In my opinion, both of them are correct. As biotechnology is a very wide area which deals with techniques of using a 'natural' organism (or its parts) as well as genetically modified organisms to produce and process useful products for mankind. A winemaker employs a strain of yeast to produce wine

by fermentation (a natural phenomenon), while the molecular biologist has cloned a gene for the antigen (that is used as a vaccine) in an organism, which allows the production of the antigen in large amounts.

**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:** The experiment is not likely to be affected as the recombinant DNA molecule is circular and closed, with no free ends. Hence, it will not be a substrate for the exonuclease enzyme, which removes nucleotides from the free ends of DNA.

**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:** If the restriction enzymes cut DNA at random sites instead of at specific sites, then the DNA fragments obtained will not have 'sticky ends'. In the absence of sticky ends, the construction of a recombinant DNA molecule 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.**

**Ans:** When a plasmid DNA and a linear DNA having one site for a restriction endonuclease are cut and separated, the plasmid shows one DNA band, while the linear DNA shows two bands because of the difference in their basic structure. A plasmid is a circular DNA molecule, and when cut with these enzymes, it becomes linear but does not get fragmented due to the presence of only one restriction site, whereas a linear DNA molecule gets cut into two fragments.

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

**Ans:** A compound called ethidium bromide stains DNA, which, on exposure to ultraviolet (UV) radiation, gives an orange light band of DNA. Hence, DNA fragments appear as an orange band in the presence of ethidium bromide and UV light.

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

**Ans:** In a gene cloning experiment, first a recombinant DNA molecule is constructed, where the gene of interest is ligated to the vector (the step would not be affected) and introduced inside the host cell (transformation). Since not all the cells get transformed with the recombinant/plasmid DNA, in the absence of a selectable marker, it will be difficult to distinguish between transformants and non-transformants, because the role of the selectable marker is in the selection of transformants.

**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:** The reasons are as follows

- (i) The DNA sample that was loaded on the gel may have been contaminated with nuclease (exo or endo, both) and completely degraded.

- (ii) Electrodes were put in opposite orientation in the gel assembly, with the anode towards the wells (where the DNA sample is loaded). Since DNA molecules are negatively charged, they move towards the anode and hence, move out of the gel instead of moving into the matrix of the gel.
- (iii) Ethidium bromide was not added at all or was not added in sufficient concentration, and so DNA was not visible.

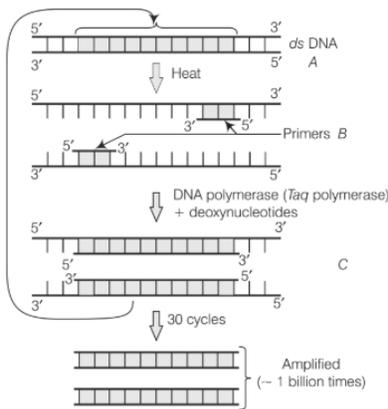
**9. Describe the role of CaCl<sub>2</sub> in the preparation of competent cells?**

**Ans:** CaCl<sub>2</sub> is known to increase the efficiency of DNA uptake to produce transformed bacterial cells. The divalent Ca<sup>+2</sup> ions create transient pores on the bacterial cell wall by which the entry of foreign DNA is facilitated into the bacterial cells.

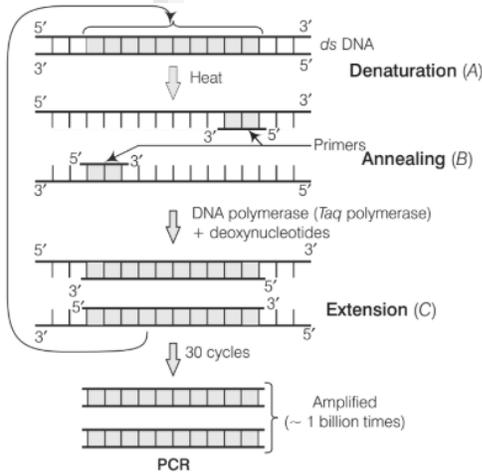
**10. What would happen when one grows a recombinant bacterium in the bioreactor but forgets to add antibiotic to the medium in which the recombinant is growing?**

**Ans:** In the absence of an antibiotic, there will be no pressure on recombinants to retain the plasmid (containing the gene of our interest). Since maintaining a high copy number of plasmids is a metabolic burden to the microbial cells, it will thus tend to lose the plasmid.

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



**Ans:** Region to be amplified

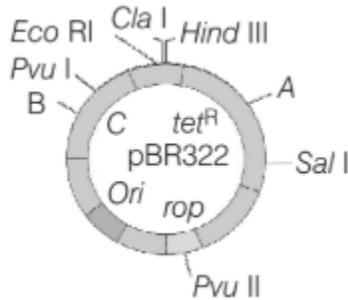


part Notes

In PCR, each cycle has three steps

- (i) Denaturation of the DNA Sample: Unwinding of the two strands of DNA by heating the sample at 92-94°C.
- (ii) Primer Annealing Primers get positioned on the exposed nucleotides as per base pairing rules.
- (iii) Extension of Primers. DNA polymerase recognises primers as 'start' tags and begins to extend the primers using the free nucleotides provided in the reaction and the genomic DNA as template. With each round of reactions, the DNA doubles.

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



**Ans:** Region A Bam HI  
Region B Pst I  
Region C ampR

## Long Answer Type Questions

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

**Ans:** In the selection of recombinants due to inactivation of antibiotics, the transformed cells are first plated on the antibiotic plate, which has not been insertional inactivated (i.e., ampicillin) and incubated overnight for growth of transformants.

- For selection of recombinants, these transformants are replica-plated on a second antibiotic (say, tetracycline) plate (which got inactivated due to insertion of the gene).
- Non-recombinants grow on both the plates (one carrying ampicillin and the other carrying tetracycline) while recombinants will grow only on the ampicillin plate.
- This entire exercise is labourious and takes more time (two overnight incubations) as well.
- However, if we choose insertional inactivation of a marker that produces colour in the presence of a chromogenic compound, we can distinguish between the recombinants and non-recombinants on a single medium plate (containing one antibiotic and the chromogenic compound) after overnight growth.

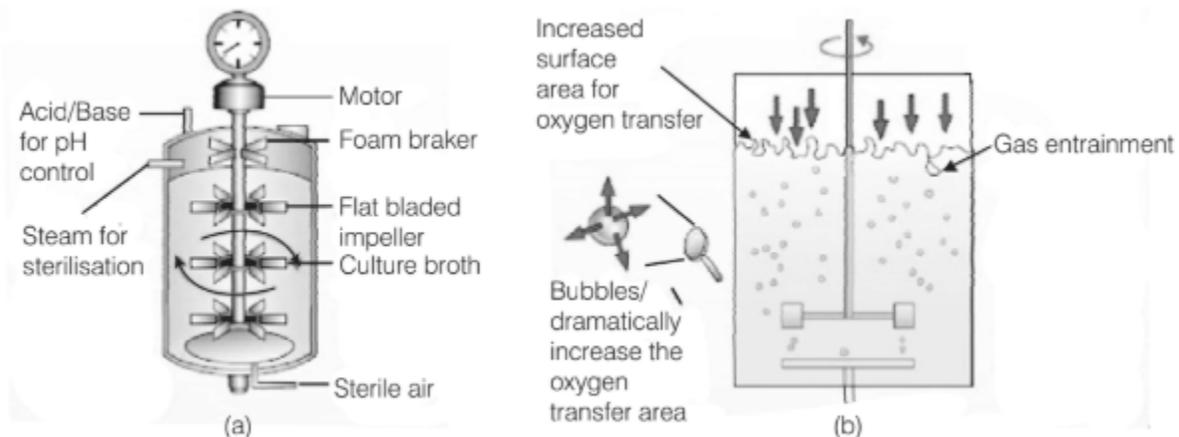
2. Describe the role of *Agrobacterium tumefaciens* in transforming a plant cell.

**Ans:** A soil-inhabiting, plant pathogenic bacterium, *Agrobacterium tumefaciens*, infects broad-leaved crops including tomato, soyabean, sunflower and cotton, but not the cereals. It causes tumours called crown galls.

- Tumour formation is induced by its plasmid, which is, therefore, called Ti-plasmid (Ti for tumour-inducing).
- The Ti-plasmid integrates a segment of its DNA, termed T-DNA, into the chromosomal DNA of its host plant cells. The T-DNA causes tumours.
- As gene transfer occurs without human effort, the bacterium is known as a natural genetic engineer of plants.
- Plant molecular biologists have started using Ti-plasmids as vectors to transfer foreign genes of interest into the target plant cells.
- They use a version of the plasmid from which the tumour-forming gene has been eliminated.
- The transformed bacteria do not cause disease, but still deliver genes of interest into a variety of plants.

**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.**

**Ans:** Bioreactors are vessels of large volumes (100-1000 L) in which raw materials are biologically converted into specific products. The most commonly used bioreactors are of the stirring type, which are shown in the figure.



- A stirred-tank reactor is usually cylindrical or with a curved base to facilitate the mixing of the reactor contents. The stirrer facilitates even mixing and oxygen availability throughout the bioreactor. Alternatively, air can be bubbled through the reactor.
- If you look at the figure closely, you will see that the bioreactor has an agitator system, an oxygen delivery system and a foam control system, a temperature control system, a pH control system and sampling ports so that small volumes of the culture can be withdrawn periodically.
- Small-volume cultures are usually employed in laboratories in a flask for the research and production of smaller quantities of products. However, large-scale production of the products is carried out in bioreactors.