Blue Print (As per PU Board)

Topic	1 mark questions	2 marks questions	3 marks questions	5 marks questions	Total Marks
Biotechnology Principles And Process	1	1	1	1	11

One mark questions

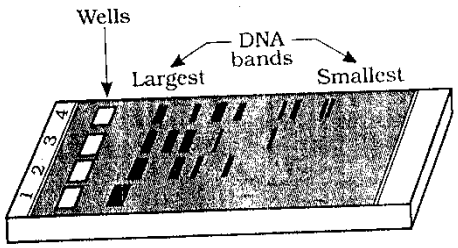
1. **What do you mean by insertional inactivation?**
   Answer: The inactivation of gene due to insertion of alien DNA is called insertional inactivation
2. **What is bioreactor?** (1 mark)
   Answer: It is a vessel in which raw materials are biologically converted into specific products using microbial plants, animal or human cells.
3. **Define plasmid?** (1 mark)
   Answer: It is circular extra chromosomal self-replicating double stranded DNA

Two marks questions

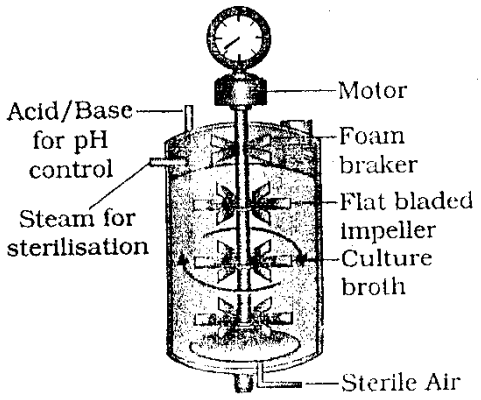
4. **What is palindromic sequences?**
   Answer: It is a sequence of base pairs that reads on the 2 sides of DNA in opposite direction (1 mark)
   Eg: 5′…GAATTC…3′ (1 mark)
   3′…CTTAAG…5′
5. **What are restriction enzymes? Mention two classes of restriction enzymes** (1 mark)
   Answer: The enzymes which are used to cut DNA at specific regions are called restriction enzymes.
   Two classes are:-
   (a) Restriction Endonucleases (REN's)
   (b) Restriction Exonucleases (1 mark)
6. **Mention the methods of making bacteria capable to take up recombinant DNA**
   Answer: (a) Calcium chloride heat treatment (1 mark)
   (b) Micro-injection

Three marks questions

7. **Write a note on Gel-electrophoresis** (3 marks)
   Answer: (1) The fragments obtained after cutting with restriction enzymes are separated by using gel electrophoresis
   (2) Electric field is applied to the electrophoresis matrix (commonly agarose gel) and negatively charged DNA fragments move towards the anode
   (3) Fragments separate according to their size by the sieving properties of agarose gel. Smaller the fragments, further it moves.



8. **Draw a labelled diagram of Bioreactor** (3 marks)
Answer: (diagram-1.5 mark & for labelling- 1.5 mark)

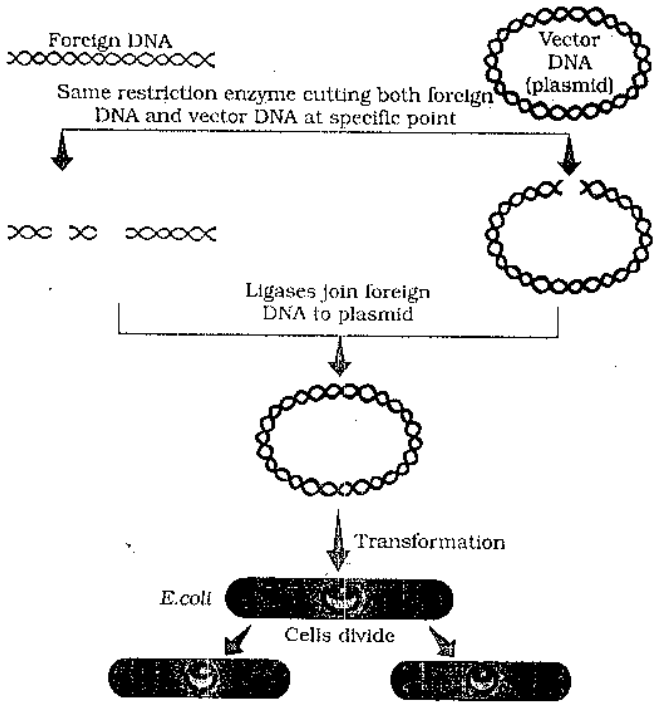


9. **Describe any three vector less methods of introducing the rDNA into a competant host cell** (3 marks)
Answer: i) Transformation: In order to force bacteria to take up the plasmid, the bacterial cell must first be made the competent to take up DNA. This is done by treating them with specific concentration of divalent cation. Eg ca^{2+} which increases the efficiency with which DNA enters the bacterium through pores in its cell wall Recombinant DNA can then be forced into such cells by inclubating the cells with recombinant DNA on ice, followed by placing them at $420°C$ & then putting them back into ice. This enables the bacteria to take up the recombinant DNA
ii) **Microinjection:-** recombinant DNA is directly injected to the nucleus of an animal cell using a micro-needle of trip with diameter (4mm)
iii) Biolistics / gene gun: Cells are bombarded with high velocity micro-particles of gold or tungsten coated with DNA.

Five marks questions

10. **Describe the various steps involved in RDNA technology with the help of a well labelled Diagram**
Answer: i) Identification of DNA with desired Genes:- other molecules in the target cell can be removed by appropriate treatment & purified DNA ultimately precipitates out after addition of chilled etnanol
ii) cutting the DNA at specific location:- After having cut the source DNA as well as vector DNA with specific restriction enzyme. The cut out "gene of interest" from the source DNA & the cut vector with space are mixed & ligase is added.
iii) Insertion of Recombinant DNA into host cell:- Recipient cells after making them competent to receive take up DNA in its surrounding. Recombinant DNA is introduced into suitable host cell by vector- based or vector-less method.
iv) selection and screening- If a recombinant DNA bearing gene for resistance to an antibiotic is transferred into E.coli in the host- cell become transformed into ampicillin. This amp r gene is called selectable marker.

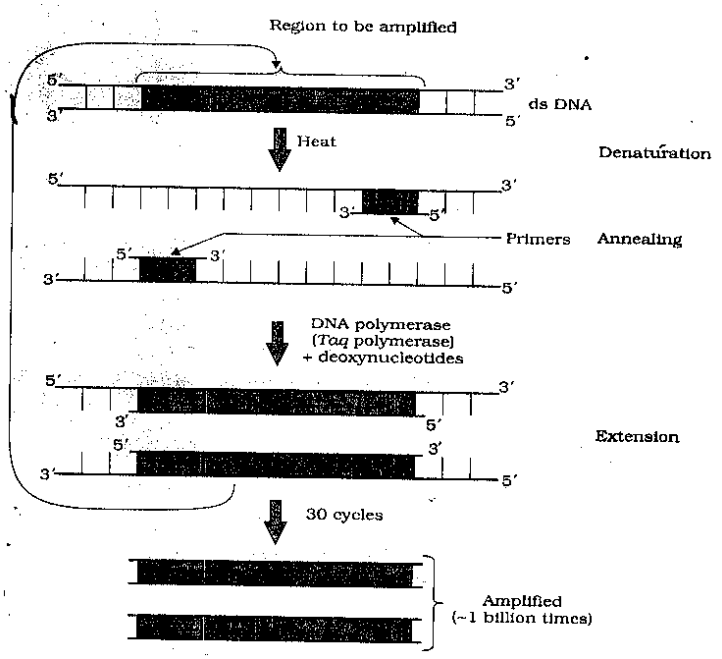
v) Obtaining the foreign Gene product:- After having cloned the gene of interest & having optimized the conditions to induce expression of the target protein, one has to consider producing it on large scale. (3 marks)



Diagrammatic representation of recombinant DNA technology (2 marks)

11. **Expand PCR? Describe the different steps involved in this technique?** (1 mark)
Answer: PCR stands for polymerase chain reaction. It is a technique for amplification of gene of interest or to obtain multiple copies of DNA of interest. The PCR requires primers, taq polymerase, target sequence DNA sample & deoxyribonulcotides. PCR includes number of cycles for amplifying DNA of interest invitro. Each cycle has three steps:-
a) Denaturation:- The first step is denaturation of SNA sample in a reaction mixture to 94°*C* during this step. DNA strands gets separated
b) Renaturation/Annealing:- The temperature is allowed to cool down to 50°*C* to allow two oligonucleotide primers to anneal to complementary sequence in DNA molecule

c) Extension:- The temperature is raised to $75°C$. At this temperature, taq-polymerase initiates DNA synthesis at 3-OH end of primer (3 marks)



(2 marks)

12. **Mention the five significance of Bio reactions** (1 mark)

Answer: 1. These are used to obtain large amounts of products. A bio reactor provides the optimal conditions (temperature, PH, substrate, salts, Vitamins. O_2) for achieving desired product. (1 mark)

2. The most commonly used bioreactors are stirring type, a stirred tank bioreactor is usually cylindrical or with a curved base made up of stainless steel to withstand repeated sterlizations. (1 mark)

3. The stirrer shaft facilitates even mixing and oxygen availability throughout the chamber. Air is bubbled through the sparger with small holes. (1 mark)

4. It has an agitator system, an oxygen delivery system, a foam control systems, a temperature control system, p^H control system & sampling ports So that small volumes of the culture and products can be withdrawn periodically. (1 mark)

5. In these tanks 100-1000 litres of culture can be processed. (1 mark)