

10801

LMDQ/D-23

RECOMBINANT DNA TECHNOLOGY

Paper : MMB-301

Time : Three Hours]

[Maximum Marks : 80

- Note :** (i) Question No. 1 is compulsory.
(ii) Attempt *one* question from each Unit.
(iii) All questions carry equal marks.

Compulsory Question

- 1.** Attempt all questions : (8×2=16)
- (i) Differentiate among PCR, RT-PCR, and qPCR?
 - (ii) What is the role of linkers, and adaptors in gene cloning experiments?
 - (iii) What is the DNA vaccine?
 - (iv) Name different reporters for protein localization in *Escherichia coli*.
 - (v) What are antisense molecules; explain with examples?
 - (vi) What is SAGE (serial analysis of gene expression)? What are its advantages?
 - (vii) List *two* important milestones in genetic engineering with their year and the scientists involved.
 - (viii) What is the advantage of using the lacZ reporter gene in yeast two hybrid experiments?

UNIT-I

2. (a) List different strategies for selection and screening of *E.coli* cells transformed by the recombinant plasmid and bacteriophage vectors. 8
- (b) What are different methods for radiolabelling of probes? Discuss their advantages and disadvantages. 4
- (c) Explain the process of Southern blotting. Why it is important to check for complete digestion of DNA with RE before Southern blotting? How to check complete transfer of DNA to the membrane? 4
3. (a) Explain the principles, procedure and importance of the following molecular biology techniques (any *four*): (4×2=8)
- (i) Southern hybridization.
 - (ii) Northern and western blotting.
 - (iii) DNA Isolation and purification.
 - (iv) RFLP.
 - (v) DNA fingerprinting.
- (b) Compare the replication fidelities of different thermostable enzymes that are being used in PCR. 2
- (c) What are genomic and cDNA libraries? How are they prepared? What are the advantages of cDNA library over the genomic library? Why bacterial cDNA libraries are rarely produced? 6

UNIT-II

4. (a) What is the Principle, Steps and Applications of STS mapping? 8
- (b) What is the difference between clone-by-clone and shotgun whole genome sequencing? Discuss different steps used in these genome sequencing methods? 8
5. Define DNA sequencing. What role does it play in genomics? How will you proceed if you have to sequence a particular fragment? Write different steps used. Explain Sanger dideoxynucleotide sequencing method. Provide an overview of performance of next-generation sequencing platforms. 16

UNIT-III

6. (a) What are recombinant proteins? Why we generally use *E.coli* as recombinant Protein Expression Host? What are different Factors affecting intracellular production of these proteins/Enzymes in *E.coli*? Also explain different types of other prokaryotic as well as eukaryotic expression systems available for the expression of heterologous proteins along with their advantages and disadvantages. 12
- (b) What is the main purpose of a DNA microarray assay? Write major steps in preparing a microarray experiment? 4

7. (a) What factors play a role in gene expression? Explain different techniques that can be used for gene expression analysis. 10
- (b) Which different techniques can be used to map transcriptional start sites? Explain at least one in detail. 6

UNIT-IV

8. What is site directed mutagenesis and for what purpose it is used for? Discuss different methods which can be used for directing site-directed mutagenesis. List some applications of site-directed mutagenesis. 16
9. What do you mean by protein-protein interactions? Why it is important to study these interactions? Discuss the utility of phage display, yeast two hybrid system and three hybrid system for studying protein-protein interactions. Which of these techniques is best and in what regard? 16
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