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JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY, WAKNAGHAT

TEST -3 EXAMINATION- 2026

M.Sc. (Microbiology) - II Semester

COURSE CODE (CREDITS): 18MS1BT313 (3) MAX. MARKS: 35

COURSE NAME: RECOMBINANT DNA TECHNOLOGY

COURSE INSTRUCTORS: Dr. Rahul Shrivastava MAX. TIME: 2 Hour

Note: *(a) All questions are compulsory. (b) The candidate is allowed to make Suitable numeric assumptions wherever required for solving problems. (c)* ***Calculators are NOT allowed.***

Q.No.	Question	Marks
Q1	A DNA molecule of 5000 bp is treated with a restriction endonuclease that has three recognition sites at positions Hind III - 500, Pst I - 1500, and ScaI -4000. Determine the number and sizes of the DNA fragments that would be obtained, when the DNA molecule is: A. Circular B. Linear	 **[2]** **[2]**
Q2	A PCR needs to be performed for the amplification of *Mycobacterium tuberculosis* Rv3291 gene. The PCR yields a specific 1670-bp product. Tm for Forward and Reverse Primers is 56°C and 58°C respectively. a. Design a PCR cycle for amplification of the 1670 bp product b. Calculate the amount of PCR product that would be obtained after 3 cycles if 12 molecules of DNA are present initially in the PCR reaction.	 **[3]** **[2]**
Q3	Compare and differentiate between genomic DNA libraries and cDNA libraries based on source material, presence of introns, representation of genes, and applications.	**[3]**
Q4	Analyze and evaluate the given situations arising during the experiments for the purification of recombinant proteins, and provide suitable solutions with reason to tackle the problem: a. If the protein that is highly unstable and tends to aggregate in *E. coli*. which tag should you use to increase the solubility and stability of your protein? b. On comparison of tags: His-Tag (6-10 AA), GST-Tag (220 AA), MBP-Tag (370	**[2 X 3 = 6]**

	AA). Which tag is least likely to interfere with the final protein's folding due to its size? c. While using the MBP-tag, why does the 'steric hindrance' make it difficult to obtain the pure target protein after the purification is complete.	
Q5	**Consider the following case studies related to DNA modifying enzymes and answer the following questions:** **A.** ***Case Study 1:*** A DNA sample was treated with two enzymes: Enzyme X removes nucleotides from the 3′ end. Enzyme Y cuts DNA at internal phosphodiester bonds. a) Identify Enzyme X and Enzyme Y as exonuclease or endonuclease. b) Which enzyme would be more useful for generating restriction fragments? Provide suitable reason. **B.** ***Case Study 2:*** A molecular biologist wants to add extra nucleotides to the 3′ end of a DNA fragment without using a template strand. a) Which enzyme should be used? b) Explain the mechanism involved. c) Mention two applications of this process in cloning. **C.** ***Case Study 3:*** A plasmid vector treated with restriction enzymes started self-ligating without accepting foreign DNA inserts. a) Which DNA modifying enzyme can be used to prevent self-ligation? b) Explain its mechanism of action. c) Why is this step important in cloning experiments?	[1] [2] [1] [1] [2] [1] [1] [2]
Q6	Write detailed Notes on following (**Any Two**): I. Applications of genetically engineered Microbes II. Biosafety Issues Related to Recombinant DNA Technology III. Yeast Two-Hybrid system to study protein-protein interactions IV. Next Generation Sequencing Technologies	**[3 X 2 = 6]**