JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY, WAKNAGHAT

TEST -2 EXAMINATION- 2025

M.Sc. (Microbiology) - II Semester

COURSE CODE (CREDITS): 18MS1BT313 (3) MAX. MARKS: 25

COURSE NAME: RECOMBINANT DNA TECHNOLOGY

COURSE INSTRUCTORS: Dr. Rahul Shrivastava MAX. TIME: 1 Hour 30 minutes

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. In a PCR experiment, the DNA template is amplified for 5 cycles. Calculate how many copies of the target DNA would be generated starting with ten DNA molecules? b. You are working with a 2,000 base pair DNA target and planning a PCR reaction using Taq DNA polymerase. How much time should be allotted for the extension step at 72°C to ensure complete amplification of your target DNA? c. You are designing a PCR protocol where the denaturation step occurs at 94°C for 30 seconds, the annealing step at 55°C for 30 seconds, and the extension step at 72°C for 1 minute. If you plan to run the PCR for 30 cycles, what will be the total time required for amplification (excluding the initial denaturation and final extension)?	**[1.5 X 3= 4.5]**
Q2	**<u>Experimental Design:</u>** You want to clone a gene encoding a medically important enzyme into an *E. coli* expression vector for large-scale protein production. The enzyme must be expressed in the correct orientation to ensure proper translation. Design an experiment to clone this gene into an expression vector, ensuring that the insert is in the correct orientation for protein production. Draw diagrams if required. Elaborate the steps and strategy to be used **under following heads,** taking suitable examples: A. Restriction enzyme selection for vector and insert.	**[2 X 4 = 8]**

	B. Ligation strategy preventing self-ligation of vector. C. Selection of recombinant cells. D. Verification of vector to contain insert.	
Q3	Expression analysis for Neem leaves (*Azadirachta indica*) and *Agrobacterium tumefaciens* need to be carried out. Which library type would you construct for each species, elaborate with justification. Design the protocol for each library type, with detailed steps. Compare the advantages and limitations of each library type.	**[6]**
Q4	Write a technical essay on 'Biosafety issues related to recombinant DNA technology' elaborating different components related to safety of personnel and environment.	**[4.5]**
Q5	Write a short note on TA Cloning **OR** TOPO TA Cloning, and its utility.	**[2]**