BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI

First Semester 2023-2024 **Mid-Semester Examination**

Date: 13-Oct-2022 | **BIO F311 Recombinant DNA Technology (Closed Book)** | Max. Marks: 70 (35%)
Time: 9:00 to 10:30 AM | Duration: 90 min

Please answer all questions in the given order. Don't jumble up the order.

Q1. List any four factors that determine the specificity and, efficiency of restriction enzyme-mediated digestion of DNA molecules. Also mention for each factor if that would increase or decrease the specificity/efficiency of the digestion with appropriate justification **[1*4=4M]**

Q2. A researcher PCR amplified "gene A" using *Pfu* DNA Polymerase. After that he has set up six different ligation reactions in six different tubes to ligate the PCR amplicon (insert) into a plasmid using blunt end ligation. The reaction conditions for all six sets are given below. Which of the following is/are expected to result in the desired ligation (cloning). **[1*6=6M]**

Justify your answer for each tube in the following format:

Tube 1: Yes/No; Reason: Because……
Tube 2: Yes/No; Reason: Because….
::::::::::
::::::::::
Tube 6:……..

Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6
Linearized plasmid with a blunt end cutter*	Linearized plasmid with a blunt end cutter*	Uncut Plasmid	Linearized plasmid with a blunt end cutter*	Linearized plasmid with a blunt end cutter*	Uncut Plasmid
PCR amplified uncut gene A (Insert)	PCR amplified gene A after digestion with a blunt end cutter*(Insert)	PCR amplified gene A after digestion with a blunt end cutter* (Insert)	PCR amplified uncut gene A (Insert)	Digested PCR amplified gene A after digestion with a blunt end cutter* (Insert)	PCR amplified gene A after digestion with a blunt end cutter* (Insert)
T4 DNA Ligase	T4 DNA Ligase	T4 DNA Ligase	*E coli* DNA ligase	*E coli* DNA ligase	*E coli* DNA ligase
Ligase Buffer containing ATP	Ligase Buffer containing ATP	Ligase Buffer containing NAD+	Ligase buffer containing NAD+	Ligase Buffer containing ATP	Ligase Buffer containing ATP

(*Note: Blunt end cutter above refers to the restriction enzyme that generates blunt ends after digestion)

Q3. What is a denaturing gel? How is it different from native gel? Why would anyone want to use denaturing gel? Specify applications of the denaturing gel. **[3*2=6M]**

Q4. List five crucial factors one must consider while designing the PCR primers with appropriate justification. **[1*5=5M]**

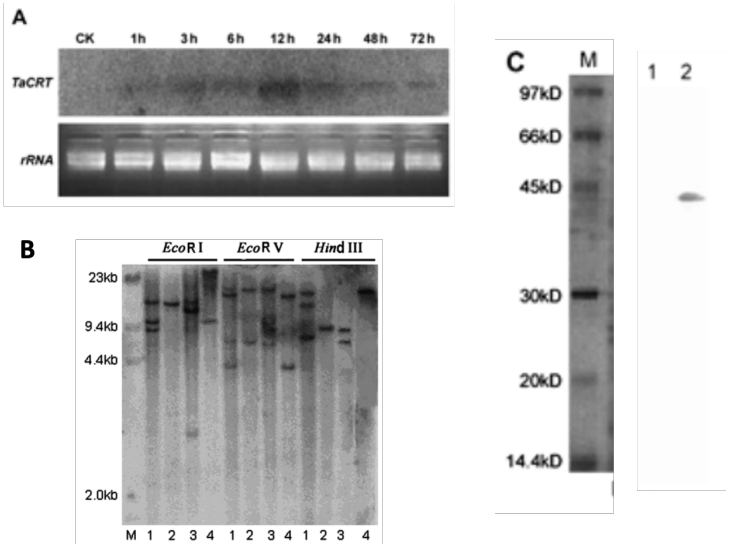
Q5. Describe two strategies that one can use to determine if the result of PCR amplification in quantitative real time PCR is specific (resulted in amplification of only desired fragment).
[2*2.5=5M]

Q6. What is the role of EtBr in CsCl-EtBr density gradient centrifugation during plasmid purification? **[4M]**

Q7. If you were to conduct an RT-PCR to detect the viral infection in a patient. List the reagents you would need to do the PCR. How are these different from reagents required for an inverse PCR? **[2+2=4]**

Q8. Given below is a figure from a published paper (Jia et al, 2008). The paper reports characterization of transgenic wheat plants containing *TaCRT* gene and corresponding protein using Southern, Northern and Western hybridization. After careful analysis of the figures below, answer following questions:

a) Identify if Figure A is result of Southern, Northern, or Western hybridization with appropriate justification. Justify the use of rRNA in this experiment.
b) Identify if Figure B is result of Southern, Northern, or Western hybridization with appropriate justification. Justify the use multiple restriction enzymes in this experiment.
c) Identify if Figure C is result of Southern, Northern, or Western hybridization with appropriate justification. What does band in lane 2 of figure C represent? **[3*4=12M]**



Q9. Would you use direct or indirect autoradiography to detect hybridization signals for S^{35} labeled oligonucleotide probes? Justify. **[4M]**

Q10. Would hybridization using 6X SSC (Saline-Sodium Citrate) be considered more stringent than 4X SSC? Give appropriate justification. **[4M]**

Q11. A) Arrange following vectors in increasing order of their insert capacity:
BAC, Plasmid, Cosmid and Bacteriophage

B) How is a cosmid different from a plasmid?

C) What are two major advantages of M13 vectors over lambda vectors? **[3*2=6M]**

Q12. A) What do clear vs. turbid plaques indicate? What kind of plaques would you expect from a temperate phage? **[4M]**

B) Suggest if a phage would go into lysogenic or lytic mode if a) CI is mutated; b) CII is mutated; c) Phage-infected bacteria are exposed to UV light. Give appropriate justification **[3*2=6M]**

Good Luck!