BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI (RAJ.)

First Semester 2022-23 BIOF311 Recombinant DNA Technology

28-Dec- 2021 (FN) **Comprehensive Examination**

Max. Marks: 40 **Paper-I (Closed-book)** Duration: 1.5 hour

Section A

Q1. Detail two techniques to separate polyadenylated RNA from other RNA's for library preparation. **[3M]**

Q2. Mention two major disadvantages which are faced when synthesizing cDNA by the self-priming method. **[2M]**

Q3. How can homopolymer tailing be used along with incorporation of restriction enzyme sites to facilitate cDNA synthesis and cloning. Explain using diagrams. **[3M]**

Q4. What is the reason for making a randomized library? Briefly explain the procedure. **[4M]**

Q5. What is the differential hybridization approach? Explain briefly, emphasizing the objective of the same. **[3M]**

Q6. Explain the concept behind "panning." **[2M]**

Q7. What is a promoter-probe vector? Explain its uses **[3M]**

Section B

Q8. How would the mutations a) *cI*, b) *cII* and c) *cIII* impact the fate of a bacteriophage after infection of the bacterial host? Justify **[1+1+1=3M]**

Q9. Sanjay used bacteria and insect cells to express the protein of his choice. In bacteria, the expression of protein was very low. In insect cells, the expression of protein was high, but the protein was not functional. Why do you think the expression of protein in bacterial system was low? Why was the protein expressed in insect cells not functional? What can he do to deal with both these challenges? **[2+2+2=6M]**

Q10. Soumya used an *E coli strain* for her experiment with genotype *end*A1 *hsd*R17 *thi*-1 *lac*Z. (Note: The *thi* mutation leads to blocking of thiamine biosynthesis while *lac*Z mutation abolishes beta galactosidase activity)
What would be the implications of these mutations on her experiment? **[1.5+1.5=3M]**

Q11. During *in vitro* protein synthesis, nuclease is added to the cell lysate which is deactivated before adding template mRNA. Justify the purpose of adding nuclease and its deactivation. **[2M]**

Q12. What is usually the insert capacity of Bacterial Artificial Chromosomes (BACs)? For what applications one would prefer to use BACs over plasmids? **[1.5+1.5=3M]**

Q13. Can we use cloning vectors and expression vectors interchangeably? Explain your answer with appropriate justification. **[3M]**

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Max. Marks: 40 **Paper-II (Open-book)** Duration: 1.5 hours

Section A

Q1. Your friend Anuj prepared a genomic library. After sequencing and analysis it appeared that there were many un-alignable sequences when compared with the reference genome. Which step could have been omitted during library preparation which may have resulted in these wrong sequencing configurations and why? **[3M]**

Q2. While making cDNA using RNase H methods, your friend forgot to add the oligo dT primer. Would he succeed in making cDNA? Explain with reasons. **[3M]**

Q3. Why is some amount of sequence information of the RNA required for performing 5' RACE? **[3M]**

Q4. While performing *in vitro* translation and immunoprecipitation with fractionated RNA, Deepak forgot to add labelled amino acids during the translation process. Explain with reasons whether the experiment would provide correct results. **[4M]**

Q5. While making a subtractive library utilizing the sticky end compatibility procedure, your friend Anita added the same restriction enzyme to both tracer and driver. Would she succeed in the experimentation? Please provide reasons for your answer. **[4M]**

Q6. What could happen in the dut/ung procedure of site directed mutagenesis if the final step of the procedure was performed in a **ung-** host ? **[3M]**

Section B

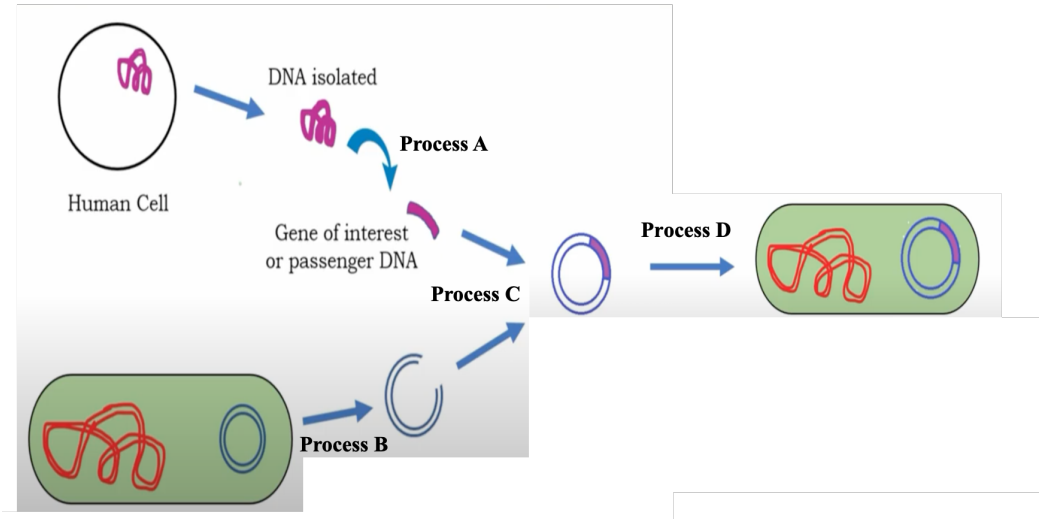
Q7. What approach/es can you use to determine the function of a gene which is

a) Important for development but not essential
b) Essential for development of an organism
c) Detrimental to the growth of the organism if the corresponding protein is expressed at high levels **[3M]**

Q8. Look at the figure below and answer the following questions:

a) What do the processes A, B, C and D correspond to?
b) How would the following affect each of the four processes if the i) Reaction mixture is incubated at 95°C before starting the process; ii) No enzyme is available for the reaction and iii) the reaction is

stopped within 10 minutes after the start of the process. Write the impact of these factors in a tabular form as shown below with appropriate justification.



Process	**Impact of incubation at 95°C**	**Impact of no enzyme available**	**Impact of stopping reaction within 15 min**
Name of Process	Rctn will still occur/not occur because	Rctn will still occur/not occur because	Rctn will be unsuccessful/partially successful because

[12M]

Q9. You regenerated three plants using recombinant DNA technology. All three were independently transformed with same transgene, vector and transformation method.

a) How would you confirm if all or some of these three plants contain the transgene?
b) Assuming all three were transgenic, how would your determine the copy number of transgene in each of them?
c) How would you determine the expression level of the transgene (at RNA level) in these three plants?
d) How would you determine the expression level of protein corresponding to the transgene in all three plants?
e) Would you expect to get same level of RNA and protein expression in all three transgenic plants? Justify. **[5M]**