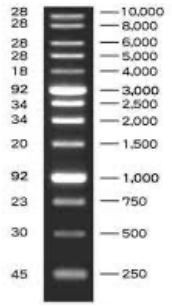
BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE, PILANI

First Semester 2023-2024 **Comprehensive Examination**

Date: 15-Dec-2023 **BIO F311 Recombinant DNA Technology (Open Book)** Max. Marks: 80

Each question carries 4 marks. Please don't jumble up the order. Write to-the-point answers with ***proper justification****!*



Q1. What does the given picture on left and numbers on both sides of the image depict? Why is the intensity of bands of different lengths different?

Q2. In a promoter probe vector, an unknown promoter was inserted in the multiple cloning site before the GUS reporter gene. A very low-level expression of the GUS gene was detected at room temperature but when incubated at 40°C, very high levels of GUS protein were detected. How would you interpret these results in terms of the capability of the promoter? Can this promoter be used for high-throughput expression of a foreign protein using *E. coli* as a host? Justify.

Q3. Given picture on the left shows long transparent strands in a solution. Your friends claim it is either genomic DNA or mRNA suspended in water. Do you think they might be right? Justify.

Q4. Does Taq Polymerase exhibit template-independent DNA Polymerase activity? Justify.

Q5. How would it impact Southern hybridization if changes in temperature and salt concentration did not have a significant impact on DNA hybridization?

Q6. How would it impact the screening strategy for identifying recombinant phages if red and gum genes were essential for the survival of viruses?

Q7. RT-PCR test was widely used to diagnose coronavirus during the recent pandemic. **A)** With appropriate justification, explain if qPCR could be used instead of RT-PCR for disease diagnosis. **B)** Which technique would you have chosen for disease diagnosis if coronavirus was a DNA virus and why?

Q8. A) A researcher forgot to prepare stacking gel for SDS-PAGE. Specify how it would impact the resolution of proteins on gel and why. **B)** The protein he was trying to resolve was tagged with a GST tag to facilitate protein purification. This tag is typically removed once protein purification is done. Assuming that the GST tag was inserted at the N-terminal end of the protein, how would it impact protein function if it could not be removed efficiently, and why?

Q9. A) A researcher is working on a genome sequencing project. He decided to use DNase for digesting genomic DNA for sequencing instead of restriction enzymes. How would it impact the genome coverage and length of sequencing reads? **B)** Next, he decides to use Illumina sequencing platform for sequencing the genomic fragments. Knowing the size limit of sequencing reads in Illumina sequencing, how would he enrich and select the fragments of desirable length for sequencing?

Q10. **A)** A researcher used banana plants as host to express a vaccine antigen. He could confirm the optimal antigen expression level in the fruits and their ability to elicit immune response using animal studies. He proposes large-scale plantations of genetically modified

banana plants and distribution of fruits for large-scale vaccination campaigns. However, the research board rejected his proposal. What do you think might be the reason for rejection? **B)** Your friend tells you that the researcher had used lipofection to transform banana plants. What steps the researcher would have followed if what your friend is suggesting is true?

Q11. A researcher tried amplifying a 2 kb fragment from genomic DNA using PCR. But despite multiple repetitions, he is getting only a single band of 1.8 kb instead of 2 kb. **A)** Give two possible reasons for this discrepancy. **B)** What can he do to determine which of the two reasons you mentioned above is the actual cause of the discrepancy?

Q12. Soumya used an *E coli strain* for her experiment with genotype *end*A1 *Kan*^{R} *thi*-1 *lac*Z. **A)** Justify if she can use kanamycin as a selection marker to screen for bacteria transformed with the recombinant plasmid. **B)** Justify if she can use LacZ as an insertion marker to screen for bacteria transformed with recombinant plasmid.

Q13. Sixty people died in a plane crash while forty were missing. **A)** Which recombinant DNA technology can be used to identify the people who have died from the remains recovered from the crash site and why? **B)** Further investigation revealed that the pilot had ingested some herb that made him lose control. A container of leaf samples could be recovered from his pockets, but researchers have no clue which plant species they might belong to. Which technique can be used to identify the origin of leaf samples and place them in the appropriate taxonomic category?

Q14. A researcher was doing Sanger sequencing but she ran out of specific DNA Polymerase purchased for Sanger sequencing. The new shipment would take a month, so she decided to purify polymerase in the lab from the appropriate host organism. **A)** Explain if she can simply purify the polymerase and use it for sequencing or if she needs to make certain changes so that it is suitable for Sanger sequencing and why? **B)** Which technique have you studied in this course that can be used to make the changes specified above?

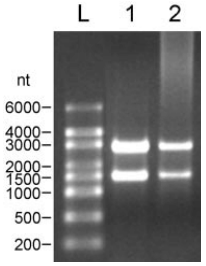
Q15. Oligo synthesis using the phosphorimidite method is a cyclic process. **A)** How many times would the cycle need to be repeated to generate a primer of 20 bp, and why? **B)** How is it ensured that no errors are introduced during the primer synthesis?

Q16. How would it impact the result of yeast two-hybrid assay **A)** If the DNA binding domain is mutated? **B)** If the bait protein is a membrane-localized protein?

Q17. How is the natural affinity of streptavidin for biotin utilized in recombinant DNA experiments? Give two examples.

Q18. Lactoglobulin is one of the principal milk proteins. **A)** Why do you think researchers use lactoglobulin promoters for recombinant protein expression in mammalian hosts? **B)** How would it impact the experiment if they use tetracycline-inducible promoter instead of lactoglobulin promoter for recombinant protein expression?

Q19. A researcher isolated genomic DNA, RNA and cDNA on the same day and forgot to label the tubes. He runs one of the samples on gel and obtains the pattern shown in the picture on the left. With appropriate justification, explain if this possibly is genomic DNA, RNA, or cDNA. Give reasoning for each case; why or why not it is genomic DNA, RNA, or cDNA?



Q20. List two advantages of Oxford Nanopore sequencing over PacBio and Illumina sequencing platforms (These features should be unique to Oxford Nanopore)