Birla Institute of Technology and Science, Pilani – Hyderabad Campus Semester I, 2022-23 rDNA Technology (BIO F311) Comprehensive Examination Date: 20.112.2022 PART A (Closed Book) + Part B (Open book) Marks: 70 (Part A 35M + Part B 35M)

Time 9:30 to 12:30 AM Duration: 180 Min (Part A 90 mins + Part B 90 mins)

Student Name:

Student ID:

Read the instructions carefully

- 1. Must write your name and ID in the indicated space over question paper.
- 2. <u>Part B (Open book) only study material (notebook, text book, PPT print) with</u> <u>bind form be allowed</u>
- 3. Write your answers legibly in the space given in the indicated box at the end with own language. Direct copy from the PPT or textbook will not be allowed.
- 4. No overwriting is allowed.
- 5. Use <u>only pen</u> to write in the respective boxes.

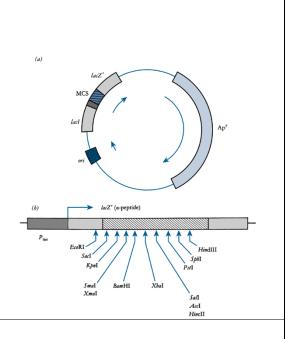
Part B Open book (35 Marks Duration 90 mins)

- If a gene produces a pre-mRNA that is 1200 base pairs long and has the following intron-exon structure: Exon 1 - 200 bp, Intron 1 - 100 bp, Exon 2 - 50 bp, Intron 2 - 150 bp, Exon 3 - 700 bp. During alternative splicing pre-mRNA skips Exon 2. How many base pairs long would we expect the mature mRNA to be? Show your calculation. 2M
- After sequencing of a nucleic acid you found the percentage of bases A 21, T 29, G21, C 29 respectively. Can you tell what kind of genetic material it is? Justify your answer. 2M
- Can hot start polymerase improve PCR reaction? how?
 How it is different from the PCR method invented by Kary Mullis? 2 + 2 = 4M

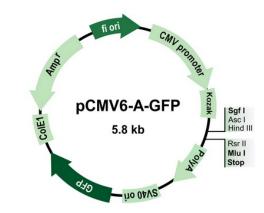
4.

A gene of insert (1800 base pair (bp) long) has been cloned in the BamHI site of a vector of ~3200bp size. The length between Bam HI and EcoR I is 500 bp. After cloning you did a double digestion with Bam HI and EcoR I. (Note GOI has a EcoRI site at 100 bp after the start codon). After successful cloning and double digestion with Bam HI and EcoR I when you will run it in the gel what band size would you expect?

- **A.** With diagram explain how would your gel will look like? Also draw the restriction map. **5M**
- **B.** How do you select the positive clones? Explain with proper justification. **5M**



5. Below is an expression vector



If you able to clone human actin gene in the indicated MCS then what will happen in terms of expression of the genes present in the above vector when you are propagating the inside E. Coli, in human embryonic kidney (HEK) cells and budding yeast? Justify your answer. **3M**

What is the benefit genomic DNA library over cDNA library? **2M**

6.

- A. Primers are generally very specific to amplify some specific target gene. But low stringy primers are very useful in some context. Can you identify in which case it will be useful and how will this help? **5M**
- B. What are the components required for the PCR having low stringy primers? 2M

- 7.
- A. Amit wants to do a site directed mutagenesis but he cannot buy Dpn I enzymes which will be helpful for the mutagenesis. Can you suggest an alternative way of mutagenesis? Diagrammatically show the how can it be performed? **2.5M**
- B. How the following chemicals (Formic acid; Dimethyl sulphate; Hydrazine, sodium chloride) is useful with respect to sequencing of gene X? **2.5M**

