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)

(Set-A)

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. **Part A (closed book) question paper will be collected after 90 mins.**
3. **Write your answers legibly in the space given in the indicated box at the end.**
4. **No overwriting is allowed.**
5. **Use only pen to write in the respective boxes.**

Part A closed book (35 Marks Duration 90 mins)

1.
   A. Mention the name of the two enzymes with opposite function required for cloning. Describe their function. **1M**
   B. During mRNA isolation somehow you lose all the polyA tails of your mRNA population. Can you able to synthesize cDNA? How? **2M**
   C. Describe with diagram any one method of blunt end cloning? **3M**

2. Given the DNA strand: **5' -TCTTGTAATTGACGTCGGAAT -3',**

   A. The complementary strand is _____(a)_____. If the complementary strand is the template, the mRNA reads as_____(b)_____ . If the above

mRNA is used for translation, the amino acid sequence is _____(c)_____.

B. What is the open reading frame (ORF) of the **given DNA strand**? Give justification with the definition of the ORF.

B. Can you tell which biological process occurs against central dogma? Explain with an example.

D. What is satellite DNA? Mention a technique that can separate the satellite DNA. (**0.5 + 0.5 + 1 + 1.5 + 1.5 + 1 = 6 M)**

3. Amit was discussing with Tanay about the nucleic acid structures. Amit was referring nucleic acid (a) having the methylated nucleotide “y”. But the unmethylated form of y (nucleotide “x”) is one of the building blocks of the nucleic acid (b) replacing the nucleotide y. Amit mentioned to Tanay that “the “y” nucleotide has a 3’ phosphate group to make phosphodiester bond”. Tanay said Amit that it is not at the 3’ position. Based on this discussion can you tell the following?
   A. Can you identify the nucleotide “y” and “x”? **0.5+0.5 = 1M**
   B. What is the difference between nucleotide and nucleoside? **1M**
   C. Can you identify the nucleic acid “a” and “b” respectively? **0.5M**
   D. What is the sugar component of the nucleotide in the “a” and “b” nucleic acid respectively? **0.5M**
   E. Using a spectrophotometer Amit and Tanay differentiated the two nucleic acid’s identity and purity. Can you tell how did they do that? **1.5M**
   F. Imagine Amit and Tanay want to know the sequence of a gene “U”. For that Amit cloned gene U using nucleic acid “a” whereas Tanay used the nucleic acid “b” to clone the same gene “U” using appropriate procedures. What different information can be obtained from the two different nucleic acid forms? Explain **1.5M**

4. Difference between gene replacement vs gene addition therapy? gene addition therapy cannot be used in which condition? **1.5M**

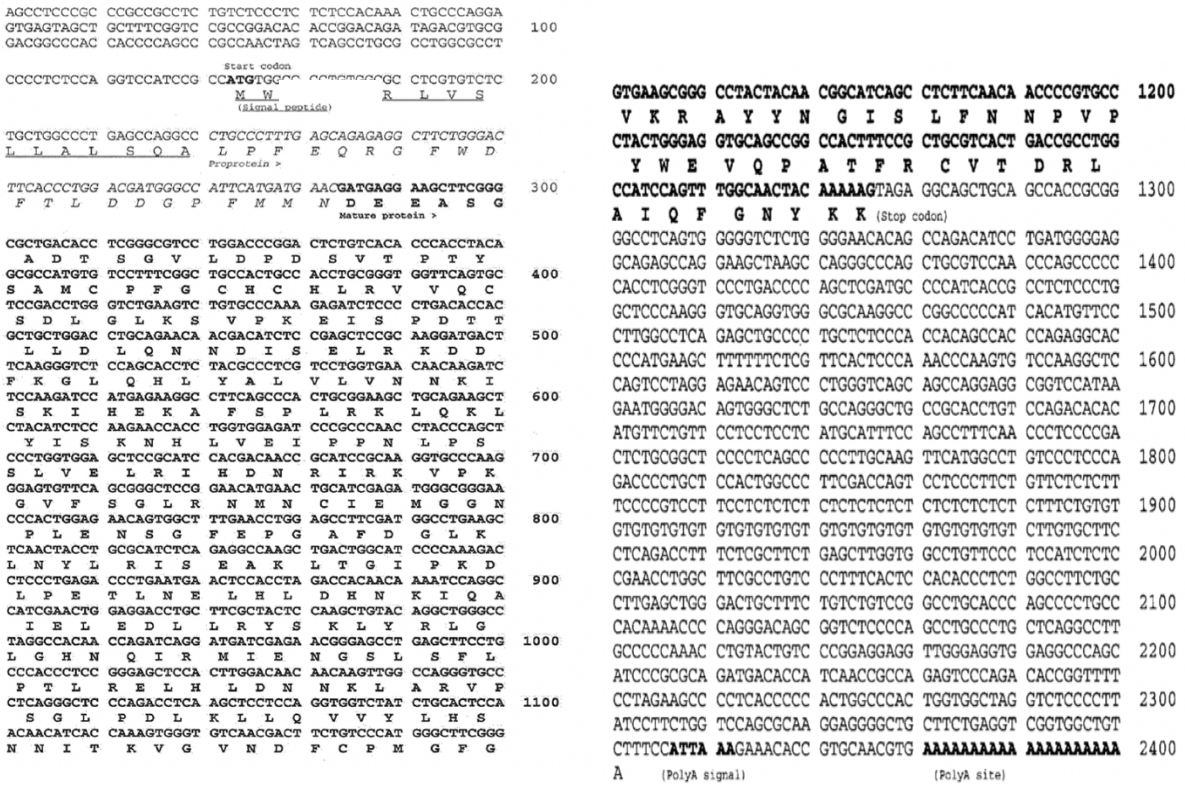
5.

A. Mention the differences of cloning vector and expression vector. **2M**

B. What is “Gate way” cloning and “TOPO TA” cloning? Describe any one of these two cloning procedures **only** step wise with diagram. **1M + 2M**

C. What is disarmed vector? **1M**

6. Carefully check the below sequence and give the answer



A. What could be the most probable type of sequence it is? is this from a eukaryotic organism or from a prokaryotic organism? **1M**

B. Can you identify the Kozak sequence? **0.5M**

C. If you wish to clone only the open reading from then what could be the size (bp) of your cloned portion? **0.5M**

7. We use different enzymes in different purposes during rDNA technology. Polymerases are among those which are very important in several purpose. Can you distinguish them according to their properties. **2M**

Activity	Polymerase activity (5' to 3' or 3' to 5')	Exonuclease activity (5' to 3' or 3' to 5')	DNA dependent RNA polymerase/RNA dependent DNA polymerase / DNA dependent DNA polymerase
Taq Polymerase			
E. coli DNA polymerase I			
Klenow Fragment			
Pfu DNA polymerase			

B. Mention differences between TYPE II and III restriction enzymes. **1M**

C. Describe any two methods used for labelling/end labelling DNA (clearly mention the enzymes employed in labelling and why are the enzymes used?) **3M**

8. In CRISPR/CAS9 method how the specific double stranded break has been generated? **1.5M**

GENETIC CODE TABLE