

Birla Institute of Technology and Science, Pilani – Hyderabad Campus

Semester I, 2022-23

rDNA Technology (BIO F311)

Mid Semester Examination

Date: 01.11.2018

PART A (Closed Book) + Part B (Open book)

Marks: 60 (Part A 30M + Part B 30M)

(A)

Time 9:00 to 10:30 AM

Duration: 90 Min (Part A 45 mins + Part B 45 mins)

Student Name:

Student ID:

Read the instructions carefully

1. Must write your name and ID in the indicated space over question paper. Part A question paper will be collected after 45mins.
2. Write your answers legibly in the space given in the indicated box at the end.
3. No overwriting is allowed.
4. Use only pen to write in the respective boxes.

**Part A closed book (30 Marks Duration 45 mins)**

**Short Answer Type (5 M X 6 = 25 M)**

1.

- A) DNA sequence by convention has been written on the coding strand. Why? Explain. (2M)
- B) What is an open reading frame? What is the open reading frame for the following sequence 5' -TCTTGTAATTGACGTCGGAAT. Justify. (1+2 = 3M)


2. Cystic fibrosis is an autosomal recessive disease in which the cystic fibrosis transmembrane conductance regulator (CFTR) protein is abnormal. The transcribed portion of the cystic fibrosis gene spans about 250000 base pairs of the DNA. The CFTR protein, with 1480 amino acids, is translated from an mRNA of about 6500 bases.
- a) Why is the RNA coding sequence of this gene so much larger than the mRNA from which the CFTR protein is translated? **1 M**
  - b) What percentage of the mRNA makes up the 5' and 3' untranslated regions (excluding introns) for the CFTR protein? **2 M**
  - c) If the gene CFTR is of 50000 bp with 20% Guanosine. What will be the number of hydrogen bonds in the CFTR? **2 M**

Note: Show all the steps of your calculation.

3. What is dg.dc telling in context of blunt end cloning? Explain with schematic diagram. (1+4=5M)

4. Amit is trying to perform sanger sequencing for a gene with 500bp. During setting up the PCR your friend found he has no dideoxy guanosine and dideoxy cytosine nucleotides. But he continued the reaction with all other required component along with radioactively labelled dideoxy adenosine and thymine nucleotides. During the sequencing of the 12bp sequence 3'-gcgcccgaattggc- 5' using a primer 5'cgcgggg-3'
- A) Can he able to perform the PCR? Give one line Justification. **1M**
  - B) What are the components required for a successful sanger PCR reactions? **1M**
  - C) If you think the PCR is possible , then write down the expected sequence of the PCR products. **2M**
  - D) Can Amit able to identify the sequence? Justify. **1M**



5. "TCTAATGGAGGT" is a hypothetical 12bp sequence.

Add an "ATG" to the sequence and use it as a coding strand with an appropriate directionality.

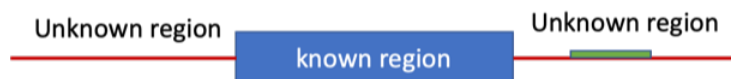
Write down double stranded structure.

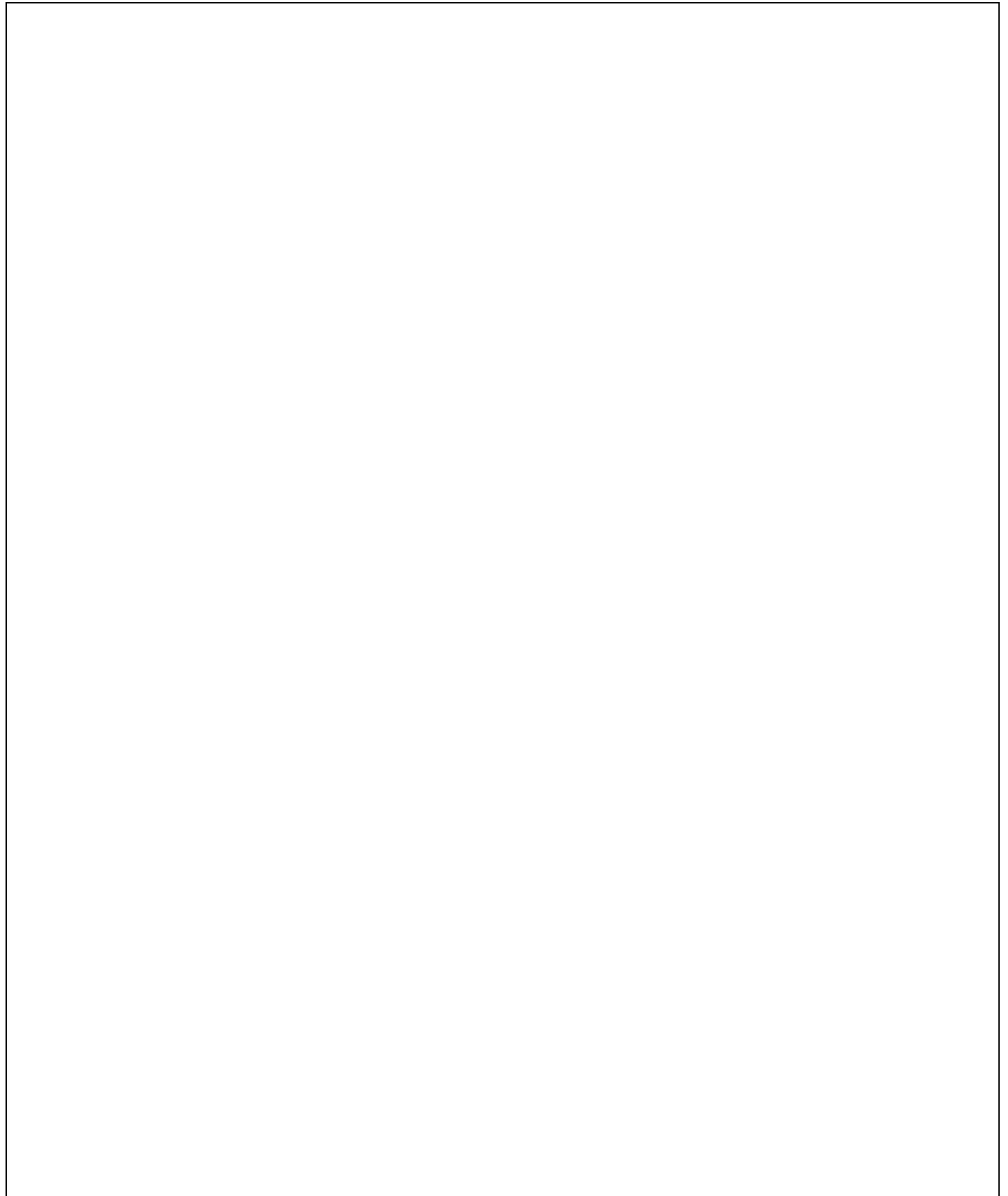
What would be the mRNA sequence and the sequence of hypothetical protein.

Note: maintain the directionality from DNA to protein sequence.

You want to clone the 15bp sequence in a plasmid. Can you draw a plasmid cloning vector (map) that can propagate in *E Coli*. (1+ 0.5 + 1 + 0.5 + 2 = 5M)

6. A stretch of DNA sequence is known, but the desired target sequence lies outside this region (like below image). What will be your PCR strategy to amplify the sequence lying in the unknown region? (5M)





# GENETIC CODE TABLE

		SECOND LETTER				
		U	C	A	G	
FIRST LETTER	U	UUU } Phe UUC } UUA } Leu UUG }	UCU } UCC } Ser UCA } UCG }	UAU } Tyr UAC } UAA Stop UAG Stop	UGU } Cys UGC } UGA Stop UGG Trp	U C A G
	C	CUU } CUC } Leu CUA } CUG }	CCU } CCC } Pro CCA } CCG }	CAU } His CAC } CAA } Gln CAG }	CGU } CGC } Arg CGA } CGG }	U C A G
	A	AUU } Ile AUC } AUA } AUG Met	ACU } ACC } Thr ACA } ACG }	AAU } Asn AAC } AAA } Lys AAG }	AGU } Ser AGC } AGA } Arg AGG }	U C A G
	G	GUU } GUC } Val GUA } GUG }	GCU } GCC } Ala GCA } GCG }	GAU } Asp GAC } GAA } Glu GAG }	GGU } GGC } Gly GGA } GGG }	U C A G