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 Mins (Part A 45 mins + Part B 45 mins)**

Read the instructions carefully

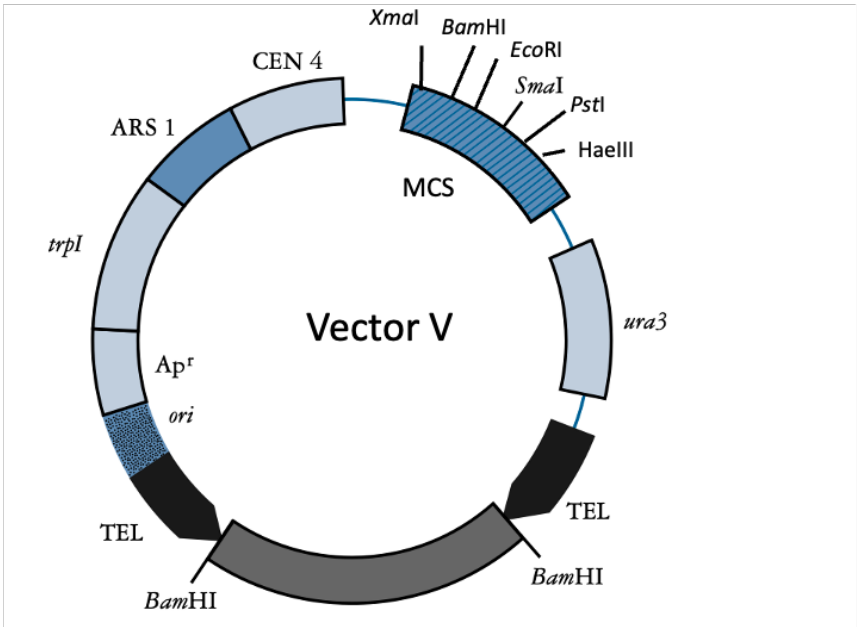
1. **Must write your name and ID in the indicated space over answer sheet and the QP set number. Without QP set number no evaluation.**
2. **Write your answers legibly in your own way. Do not copy lines from the study materials.**
3. **No overwriting is allowed.**
4. **Use only pen to write your answer.**

Part B Open book (30 Marks Duration 45 mins)

1. A hypothetical cDNA product of a mouse gene Y is written below (5' and 3' sequences are mentioned)

5'-CCGAATTCGGATTCCCGGGGTTAAC---------GGATCCACCTTGGCCAAACCCGGG-3'

You need to clone this into a vector V. The plasmid Map is below:



Carefully check the vector and the Gene of interest (GOI) to be cloned and give the answer of following questions.

a. Do you think cloning of the Gene Y (cDNA) be possible? If yes, mention any approach you think will be suitable for cloning the gene. Justify your answer and describe the method briefly. **1+3 = 4M**

b. Which restriction enzyme will be useful during the cloning of gene Y (cDNA) in the vector V? Justify. (Note details of the restriction enzyme given at the end of the question paper) **2M**

c. Which kind of vector is it? How many types of host cell(s) it can replicate? **1.5 M**

d. There are multiple genes present in vector V along with your GOI. Can you tell which gene will express in which host(s)? and why? **1.5M**

e. Imagine the host strain lacks histidine and tryptophan synthesis gene. In a synthetic media which has every essential component except tryptophan and uracil. Which of the following host strains can probably be used to select a transformants (i.e. cells harbouring the recombinant plasmid) (Note: Δ means deletion of that particular gene)

I. Mutant Strain A (*trp*1Δ0; *His*Δ0)
II. Mutant Strain B (*trp*1Δ0)
III. Mutant Strain C(*trp*1Δ0 ura3 Δ0)
IV. Mutant Strain C(*ura*3 Δ0 His Δ0)
Justify your answer. **3M**

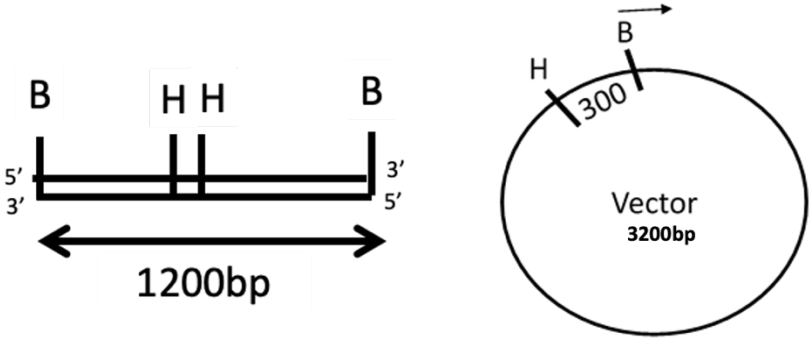
2. To amplify a gene your friend made the three following forward primers. If the reverse primer is fine then which one of the three will be the best forward primer and why? Justify your answer. (**3M**)

Forward Primer 1 : 5’ gcgcctatgaguagcggc 3’

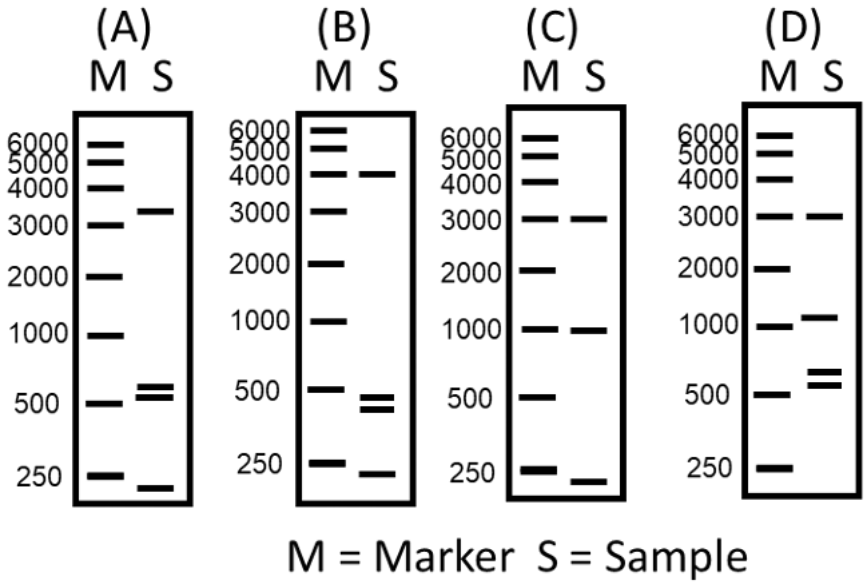
Forward Primer 2 : 5’ gcgtggatgaaagcaggc 3’

Forward Primer 3: 5’ aagcctatgagucggctt 3’

3. Given below are the maps of a 1200 base pair (bp) long DNA insert and a ~3200bp expression vector. The Bam HI (B) and Hind III (H) restriction sites and DNA length between them are indicated in base pairs. The insert is cloned in the vector at Bam HI site only and the desired orientation is shown by the arrow.



After cloning the orientation of the insert in the recombinant plasmid is tested by complete HindIII digestion followed by agarose gel run. One of the HindIII cut site is at the 600bp position. Which one of the following band patterns reveals the correct orientation of the insert in the concert? Justify **(3M)**



4. Amit and Aahan is trying to understand expression difference of Gene X between healthy cells and cancerous cells. Both of them isolate RNA and make cDNA. Amit performed conventional PCR for 30 cycles and run in electrophoresis gel and found the same intensity band of gene x in healthy cell and cancer cells (see the figure 1). But Aahan performed real time PCR and found C_t value for healthy cell 18 and C_t value 15.

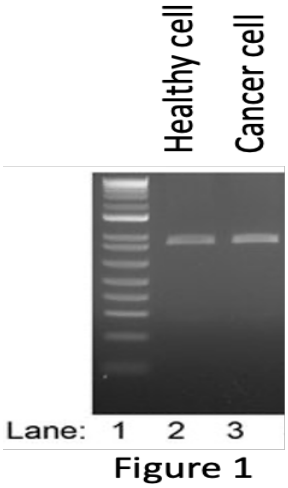


Figure 1

Based on this experiment answer the following questions

a. How cDNA was made from mRNA? Describe the process mentioning all the components. **3M**
b. What are the components used by Amit while performing conventional PCR? **1M**
c. Which components are the different in at the real time PCR? **1M**
d. What are the conclusions you can draw from the results of Amit and Aahan respectively? Explain. **3M**
e. Which results of the two different PCR is more correct? Justify your answer. **2M**
f. Suppose Amit used hot start PCR. What is hot start PCR? How it is different from the PCR method invented by Kary Mullis? **2M**

Table 2.1. The genetic code

First base (5′ end)	Second base U	C	A	G	Third base (3′ end)
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Note: Codons read 5′→3′; thus, AUG specifies Met. The three-letter abbreviations for the amino acids are as follows: Ala, Alanine; Arg, Arginine; Asn, Asparagine; Asp, Aspartic acid; Cys, Cysteine; Gln, Glutamine; Glu, Glutamic acid; Gly, Glycine; His, Histidine; Ile, Isoleucine; Leu, Leucine; Lys, Lysine; Met, Methionine; Phe, Phenylalanine; Pro, Proline; Ser, Serine; Thr, Threonine; Trp, Tryptophan; Tyr, Tyrosine; Val, Valine. The three codons UAA, UAG, and UGA specify no amino acid and terminate translation.

Table 4.1. Recognition sequences and cutting sites for some restriction endonucleases

Enzyme	Recognition sequence	Cutting sites	Ends
*Bam*HI	5′-GGATCC-3′	G↓GATCC CCTAG↑G	5′
*Eco*RI	5′-GAATTC-3′	G↓AATTC CTTAA↑G	5′
*Hae*III	5′-GGCC-3′	GG↓CC CC↑GG	Blunt
*Hpa*I	5′-GTTAAC-3′	GTT↓AAC CAA↑TTG	Blunt
*Pst*I	5′-CTGCAG-3′	CTGCA↓G G↑ACGTC	3′
*Sau*3A	5′-GATC-3′	↓GATC CTAG↑	5′
*Sma*I	5′-CCCGGG-3′	CCC↓GGG GGG↑CCC	Blunt
*Sst*I	5′-GAGCTC-3′	GAGCT↓C C↑TCGAG	3′
*Xma*I	5′-CCCGGG-3′	C↓CCGGG GGGCC↑C	5′

Note: The recognition sequences are given in single-strand form, written 5′→3′. Cutting sites are given in double-stranded form to illustrate the type of ends produced by a particular enzyme; 5′ and 3′ refer to 5′- and 3′-protruding termini, respectively. The point at which the phosphodiester bonds are broken is shown by the arrow on each strand of the recognition sequence.