

Birla Institute of Technology and Science, Pilani – Hyderabad Campus

Semester I, 2022-23

rDNA Technology (BIO F311)

Comprehensive Examination

Date: 20.11.2022

(Set-A)

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:

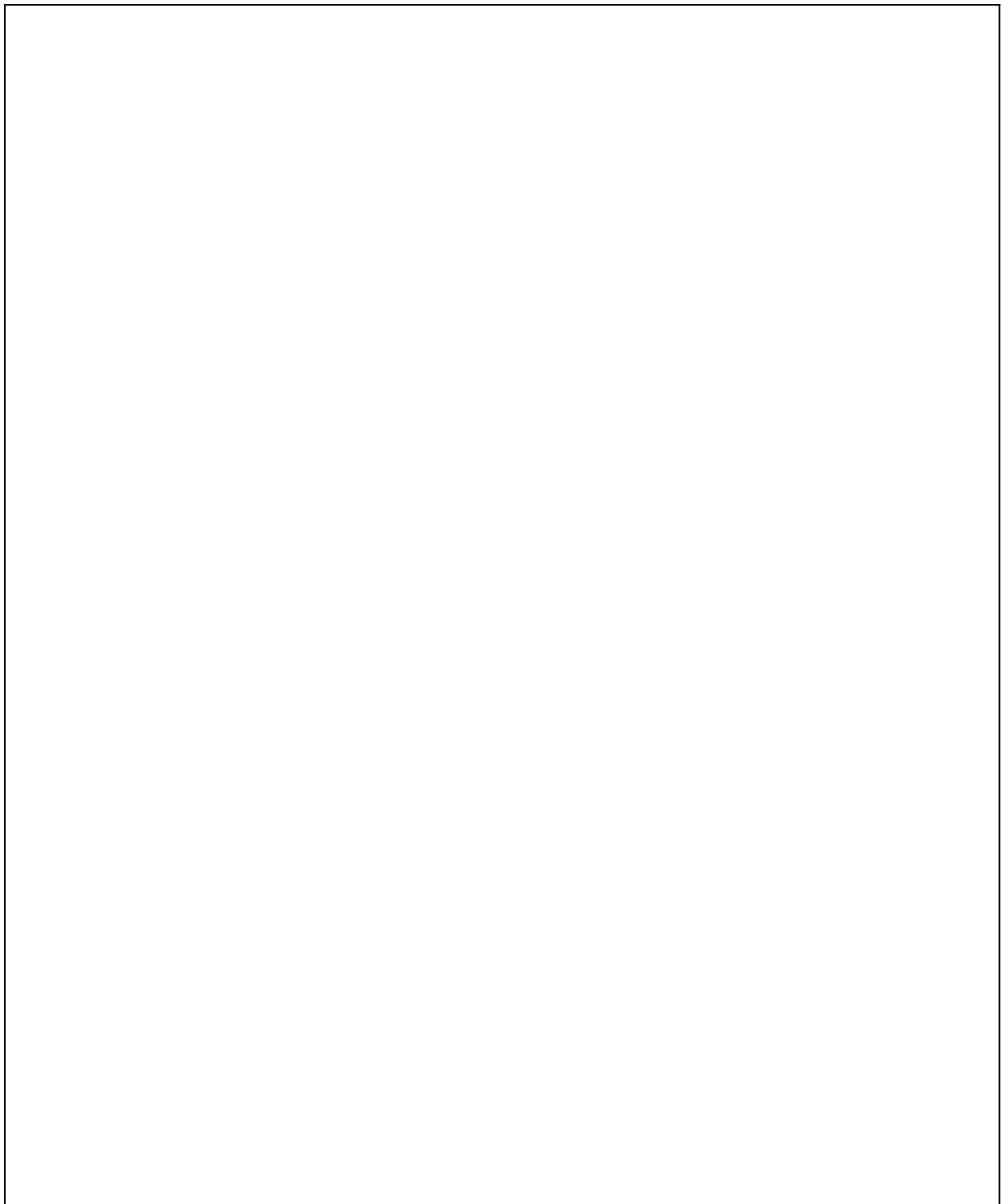
Student ID:

Read the instructions carefully

1. Must write your name and ID in the indicated space over question paper.
2. 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' -TCTTGTAAATTGACGTCGGAAT -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**

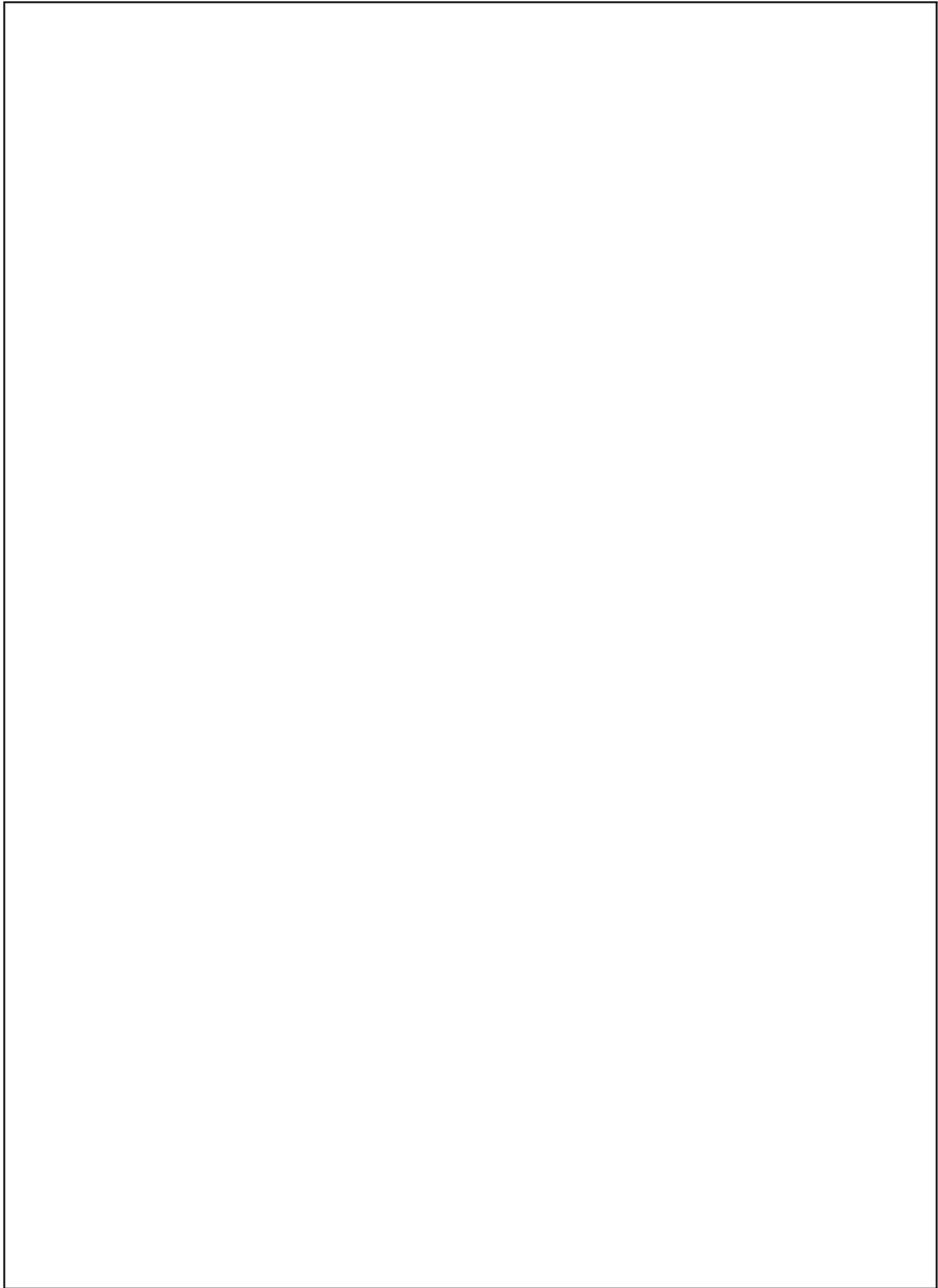
4. Difference between gene replacement vs gene addition therapy? gene addition therapy cannot be used in which condition? **1.5M**

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

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AGCCTCCCGC CCGCCGCCTC TGTCTCCCTC TCTCCACAAA CTGCCCAGGA 100
GTGAGTAGCT GCTTTCGGTC CGCCGGACAC ACCGGACAGA TAGACGTGCG
GACGGCCAC CACCCACGCC CGCCAACCTAG TCAGCCTGGG CCTGGCCGCT

          Start codon
CCCCCTCCCA GGTCCATCCG CCATGTTGGCC CCGGGCGCGC CTCTGTCTTC 200
          M W R L V S
          (Signal peptide)

TGTGGCCCT GAGCCAGGCC CTGCCCTTTC AGCAGAGAGC CTCTTGGGAC
L L A L S Q A L P P E Q R G F W D
          Proprotein >

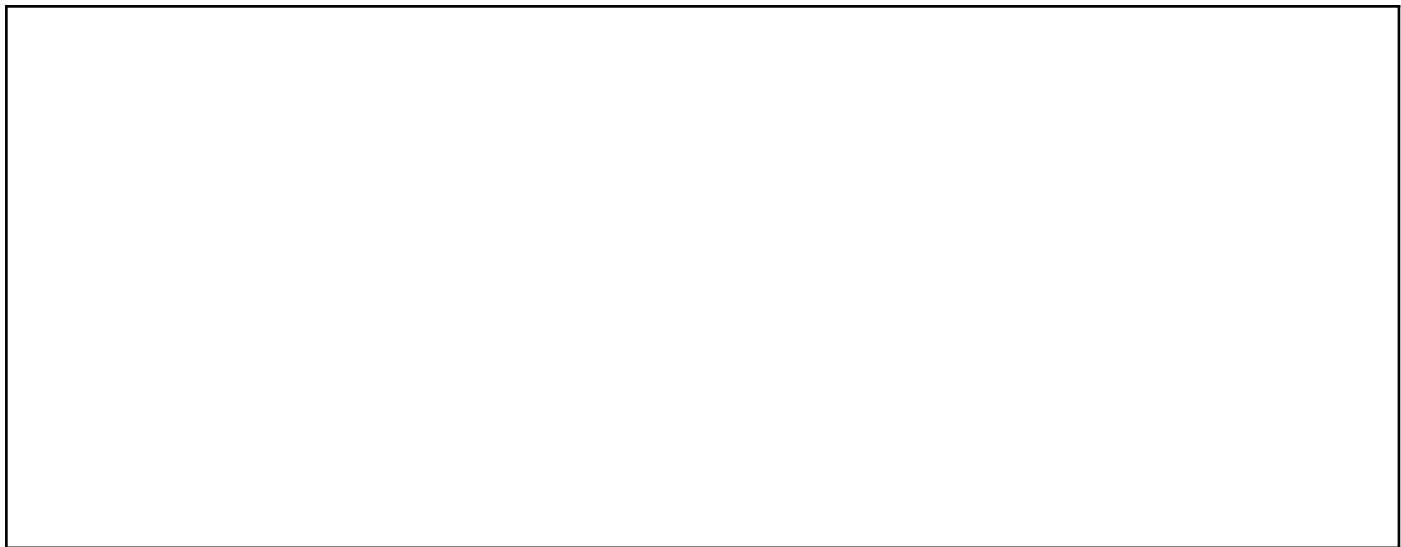
TTCACCTGG ACGATGGGGC ATTCAATGAT AACGATGAGG AAGCTTCGGG 300
F T L D D G P F M M N D E E A S G
          Mature protein >

CGGTACACC TCGGGGTCF TGGACCCGGA CTCTGTACAA CCCACATACA
A D T S G V L D P D S V T F T Y
GGCCATGTG TCCTTTCGGC TGCCACTGCG ACCTCGGGGT GGTTCAGTGC 400
S A M C P F G C H C H L R V V Q C
TCCGACTGG GTCTGAAGTC TGTGCCAAA GAGATCTCC CTGACACCAC
S D L G L K S V P K E I S P D T T
GCTGCTGGAC CTGACAGAACA ACGACATCTC CGAGTCCCG AAGGATGACT 500
L L D L Q N N D I S E L R K D D
TCAAGGTCT CCAGCACCTC TACGCCCTCG TCCTGGTGAA CAACAAGATC
F K G L Q H L Y A L V L V N N K I
TCCAAGATCC ATGAGAAGGC CTTCAGCCCA CTGCGGAAGC TGCAGAAGCT 600
S K I H E K A F S P L R K L Q K L
CTACATCC AAGAACCACC TGGTGGAGAT CCGGCCCAAC CTACCCAGCT
Y I S K N H L V E I P P N L P S
CCCTGGTGA GCTCCGCATC CACGACAACC GCATCCGCAA GGTGCCCAAG 700
S L V E L R I H D N R I R K V P K
GGAGTGTCA GCGGGCTCCG GAACATGAAC TGACATGAGA TGGCGGGGAA
G V F S G L R N M N C I E M G G N
CCCCTGGAG AACAGTGGCT TGAACCTGG AGCCTTCGAT GGCCTGAAGC 800
F L E N S G F E P G A F D G L K
TCACTACTT GCGCATCTCA GAGCCCAAGC TGACTGGCAT CCCCAGAAGC
L N Y L R I S E A K L T G I P F D
CCTCCAGAG CCTGAAAGA ACTCCACTCA GACCACACA AAATCCAGGC 900
L P E T L N E L H L D H N K I Q A
CATCGAACTG GAGGACTGCT TTCGCTACTC CAAGCTGTAC AGGCTGGGCC
I E L E D L L R Y S K L Y R L G
TAGGCCACA CCAGATCAGG ATGATCGAGA ACGGGAGCCT GAGCTTCCTG 1000
L G H N Q I R M I E N G S L S F L
CCCACCTCC GGGAGCTCCA CTTGGACAAC AACAGTGTG CCAGGGTGCC
P T L R E L H L D N N K L A R V P
CTCAGGGCTC CCAGACCTCA AGCTCCTCCA GGTGTCTAT CTGCACTCCA 1100
S G L P D L K L L Q V V Y L H S
ACAACATCAC CAAGTGGGT GTCAACGACT TCTGTCCCAT GGGCTCGGG
N N I T K V G V N D F C P M G F G

GTGAAGCGGG CCTACTACAA CGGCATCAGC CTCTTCAACA ACCCCGTGCC 1200
V K R A Y Y N G I S L F N N P V P
CTACTGGGAG GTGCAGCCGG CCACTTCCG CTGCGTCACT GACCCCTGG
Y W E V Q P A T F R C V T D R L
CCATCCAGTT TGGCAACTAC AAAAAAGTAGA GGCAGGTGCA GCCACCGCGG 1300
A I Q F G N Y K K (Stop codon)
GGCCTCAGTG GGGTCTCTG GGAACACAG CCAGACATCC TGATGGGGAG
GCAGAGCCAG GAAGCTAAGC CAGGGCCAG CTGCGTCCAA CCCAGCCCC 1400
CACCTCGGGT CCTGACCCC AGCTCGATGC CCCATCACCG CCTCTCCCTG
GCTCCCAAGG GTGCAGGTGG GCGCAAGGCC CGGCCCCAT CACATGTTC 1500
CTTGGCTTCA GAGTCCGCC TGCTCTCCA CCACAGCCAC CCAGAGGCAC
CCCATGAAGC TTTTCTCTG TCACTCCA AACCCAAGT TCCAAGGCTC 1600
CAGTCCTAGG AGAACAGTCC CTGGGTGAGC AGCCAGGAGG CGGTCCATAA
GAATGGGGAC AGTGGGCTCT GCCAGGGCTG CCGCACCTGT CCAGACACAC 1700
ATGTCTGTCT CCTCTCTCT ATGCATTTCC AGCCTTTCAA CCTCCCCGA
CTCTGCGGCT CCCCTCAGCC CCTTGAAG TTAATGGCCT GTCCCTCCCA 1800
GACCCTGTGT CCACTGGCCC TTCGACCACT CCTCCCTTCT GTTCTCTCTT
TCCCGTCTCT TCCTCTCTCT CTCTCTCTCT CTCTCTCTCT CTTTCTGTGT 1900
GTGTGTGTGT GTGTGTGTGT GTGTGTGTGT GTGTGTGTGT CTTGTGCTTC
CTCAGACCTT TCTCGTCTT GAGCTTGGTG GCCTGTTCCT TCCATCTCTC 2000
CGAACCCTGG TTCGCTGTC CTTTCACTA CACACCCTCT GGCCTTCTGC
CTTGAGCTGG GACTGCTTTC TGTCTGTCCG GCCTGCACCC AGCCCTGCCC 2100
CACAAAACCC CAGGGACAGC GGTCTCCCCA GCCTGCCCTG CTCAGGCCCTT
GCCCCCAAAC CTGTACTGTG CCGGAGGAGG TTGGGAGGTG GAGGCCCAGC 2200
ATCCCGCGCA GATGACACA TCAACCGCCA GAGTCCCAGA CACCGGTTTT
CCTAGAAGCC CCTCACCCC ACTGGCCCC TGGTGGCTAG GTCTCCCTTT 2300
ATCCTTCTGG TCCAGCGCAA GGAGGGGCTG CTTCTGAGGT CGGTGCCTGT
CTTTCATTA AAGAACACC GTGCAACGTG AAAAAAAAAA AAAAAAAAAA 2400
A (PolyA signal) (PolyA site)

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- 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**

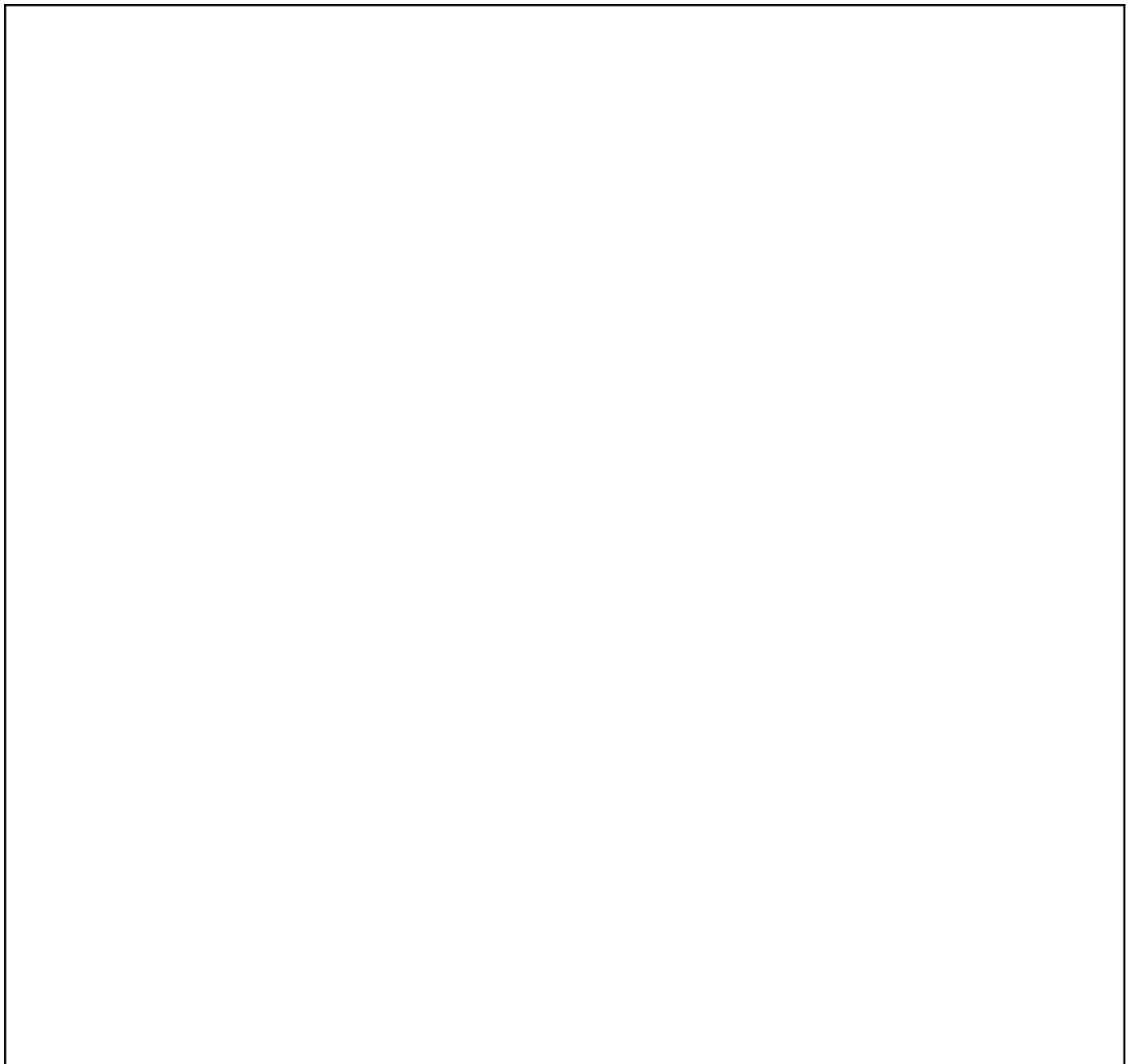


7. We use different enzymes in different purposes during rDNA technology. Polymerases are among those which are very important in several purpose. Can you distinguish them according to their properties. **2M**

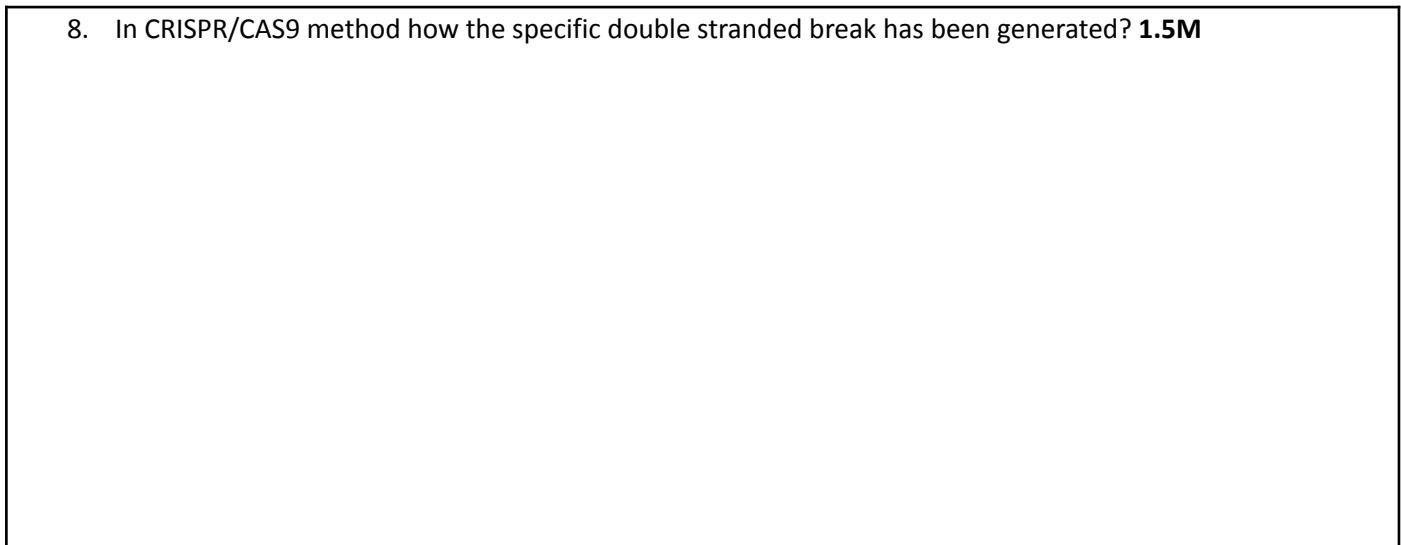
Activity	Polymerase activity (5' to 3' or 3' to 5')	Exonuclease activity (5' to 3' or 3' to 5')	DNA dependent RNA polymerase/RNA dependent DNA polymerase / DNA dependent DNA polymerase
Taq Polymerase			
<i>E. coli</i> DNA polymerase I			
Klenow Fragment			
Pfu DNA polymerase			

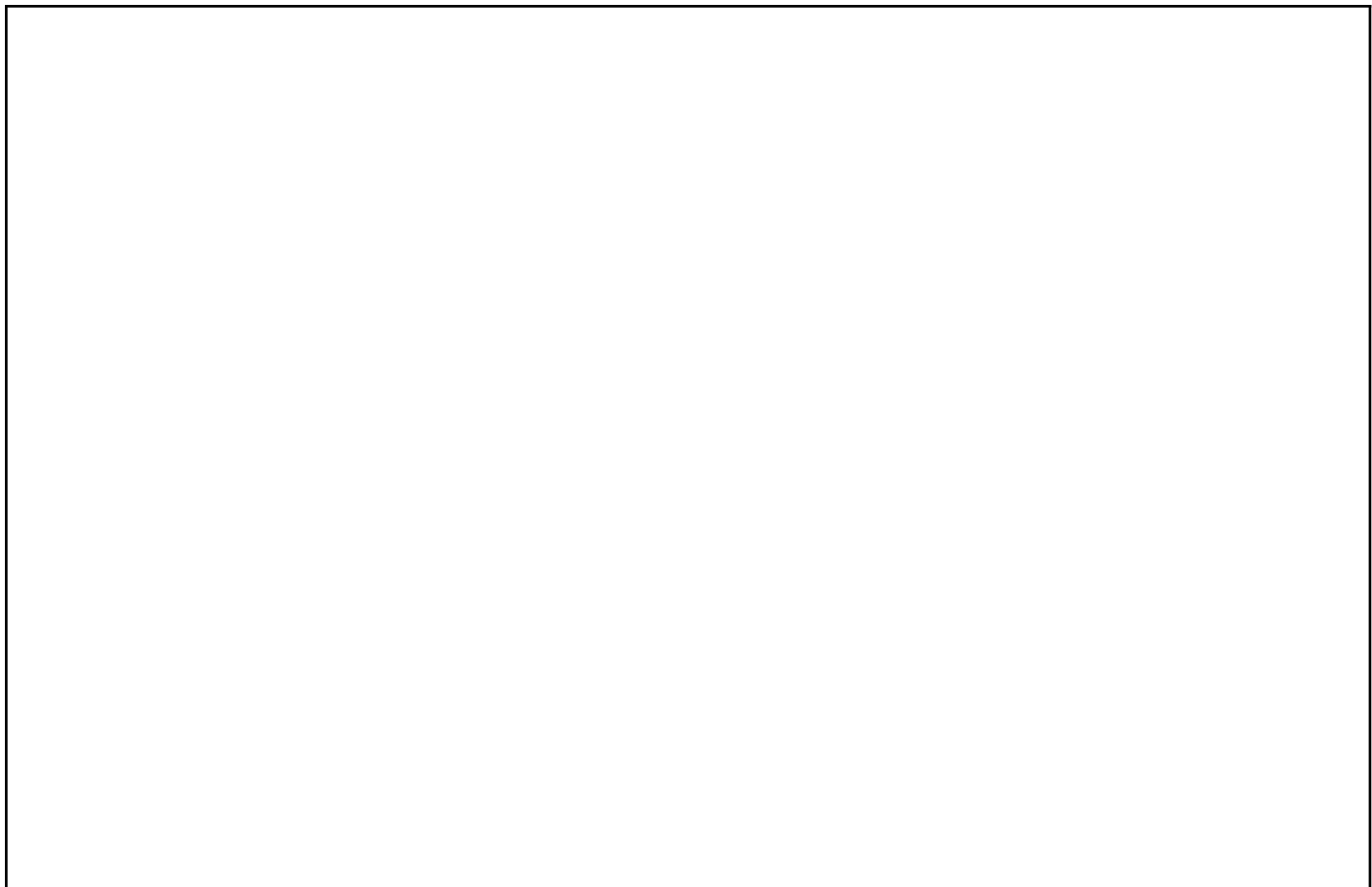
B. Mention differences between TYPE II and III restriction enzymes. **1M**

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

		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

