Name: Enrolment No:	

UNIVERSITY OF PETROLEUM AND ENERGY STUDIES
End Semester Examination, December 2022

Course: Biotechnology — Semester: IIIrd
Program: M. Sc (Microbiology) — Time : 03 hrs.
Course Code: HSMB 8003 — Max. Marks: 100

Instructions:

Q.No	Section A MCQs/Short answer questions/True &False	(20x1.5= 30 Marks)	COs
Q	Statement of question (each question carries 1.5 marks)		CO
1.	The DNA fragments have sticky ends due to a) Endonuclease b) Unpaired bases c) Calcium ions d) Free methylation	1.5	CO1
2.	Plasmid vector in DNA recombinant technology means a) a virus that transfers gene to bacteria b) extra-chromosomal autonomously replicating circular DNA c) sticky end of DNA d) any fragment of DNA carrying desirable gene	1.5	CO1
3.	How are transformants selected from non-transformants? a) Presence of more than one recognition site in the vector DNA. b) Presence of alien DNA into the vector DNA results into insertional inactivation of selectable marker. c) Antibiotic resistance gene gets inactivated due to insertion of alien DNA. d) Both 2 and 3	1.5	CO2
4.	In between which two Carbons' of consecutive sugars in a DNA molecule, a phosphodiester bond is formed? a) C1-C2 b) C2-C3 c) C3-C5 d) C2-C4	1.5	CO1
5.	Which of the following is not a characteristic of pBR322 vector? a) It is the first artificial cloning vector constructed in 1977 by Boliver and Rodriguez. b) It is the most widely used, versatile and easily manipulated vector. c) It has two antibiotic resistance genes, tet and amp. d) It does not have restriction site for Sal I.	1.5	CO2

6.	What does PCR stand for? Who is credited with the discovery of PCR	1.5	CO3
7.	State True or False: A few enzymes exhibit multiple activities that span two or more classes. e. g, many polymerases combine their ability to make new DNA molecules with an associated DNA degradative domain.	1.5	CO2
8.	With an example for each briefly explain the significance of following in in Recombinant DNA Technology: A) Vectors and B) Restriction enzymes	1.5	CO2
9.	Klenow fragment is derived from a) DNA Ligase b) DNA Pol-I c) DNA Pol-II d) d) Reverse Transcriptase	1.5	CO3
10.	Briefly explain the role of following reagents in PCR: a) dNTPs, and b) Primers	1.5	CO3
11.	Select the correct order of processing of PCR. a) Extension, primer annealing, denaturation b) Denaturation, primer annealing, extension c) Denaturation, extension, primer annealing d) Primer annealing, denaturation, extension	1.5	CO2
12.	Southern Blotting is a) Attachment of probes to DNA fragments b) Transfer of DNA fragments from electrophoretic gel to a nitrocellulose sheet c) Comparison of DNA fragments to two sources d) Transfer of DNA fragments to electrophoretic gel from cellulose membrane	1.5	CO3
13.	Plasmids are used as cloning vectors for which of the following reasons? a) Can be multiplied in culture b) Self-replication in bacterial cells c) Can be multiplied in laboratories with the help of enzymes d) Replicate freely outside bacterial cells	1.5	CO2
14.	Give an example of Restriction Endonuclease enzyme that produces: a) Cohesive ends	1.5	CO3

	b) Blunt ends		
15.	State True or False: Restriction enzymes act on "Hydrogen bonds" in a DNA molecule.	1.5	CO4
16.	Compare between linkers and adaptors?	1.5	CO4
17.	The expression of a transgene in the target tissue is identified by a a. Transgene b. Promoter c. Enhancer d. Reporter	1.5	CO3
18.	Which of the following is a palindromic sequence? a) 5'-CGTATG-3' 3'-CGAATG-5' b) 5'-CGAATG-3' 3'-GCATAC-5' c) 5'-GAATTC-3' 3'-CTTAAG-5' d) 5'-GACTAC-3' 3'-CTTAAG-5'	1.5	CO4
19.	A mixture containing DNA fragments, a, b, c and d, with molecular weights of a+b = c, a > b and d>c, was subjected to agarose gel electrophoresis. The positions of these fragments from anode to cathode sides of the gel would be a) b, a, c, d b) a, b, c, d c) c, b, a, d d) b, a, d, c	1.5	CO4
20.	Give the temperatures ranges of the following steps in a PCR? a) Denaturation b) Annealing c) Extension	1.5	CO3
	Section B	(4x5=20 Marks)	CO
Q	Statement of question (each question carries 5 marks)		
1.	(a) Draw a well labelled diagram of DNA backbone. (b) What is Transduction? Discuss the Hershey and Chase experiment carried out to validate DNA as the genetic material.	2+3	CO1

2.	a) Restriction endonucleases are referred to as "molecular scissors" in gene cloning. Justify with relevant examples. b) Is it possible for "restriction endonucleases with different recognition sequences to produce the same sticky ends." Argue with relevant examples.	2+3	CO2
3.	(a) A tetranucleotide recognition sequence (e.g., GATC) should occur once every _____nucleotides, and a hexanucleotide sequence should occur once every_____nucleotides in a genome under ideal conditions. (b) What are the two classical assumptions for these calculations? Are these assumptions valid under realistic conditions	2+3	CO3
4.	(a) What are competent cells and why are they needed in the process of cloning? (b) Compare different protocols for obtaining the competent bacterial cells?	2+3	CO4
	Section C	(2x15=30 Marks)	
Q	Statement of question (Case studies) (each question carries 15 marks)		CO
1.	A gene of interest (G1) was cloned in a cloning vector pBR322 and pUC8 series vector, after digestion of the plasmids backbone by a restriction endonuclease, PstI. After the process of transformation bacterial cells were plated on a media. a) Give a diagram (or restriction map) of plasmid pBR322 and pUC8 series vector. Highlight the advantages that pUC8 series vectors have over pBR322 b) Describe which selectable marker/ reporter gene would exhibit insertional inactivation in recombinants, where, (i) pBR322 plasmids were used (ii) pUC8 series vectors were used c) Describe the selection method for "Transformants" and "Non transformants" generated where pBR322 were used as vectors. d) Discuss what would happen in the cloning experiment employing pUC8 series vector: (i) If you forgot to add ampicillin in the media prior to plating the transformed cells? (ii) If you forgot to add the Substrate (X-Gal) in the media prior to plating the transformed cells?	15 (6+2+4+3)	CO2

	(iii) If all the colonies obtained were white. What would you interpret from this observation?		
2.	A gene of interest (G1) is to be amplified using polymerase chain reaction. The primers were designed for the amplification of G1 and the size of PCR product was chosen to be 500bp. The Tm of the primer-pair is 55° C and the GC content is 52%. a) Discuss what would happen if: i. The annealing temp of the reaction was set at 72° C ii. The extension temperature was set at 45° C b) What is the significance of "Denaturation" step in PCR? c) What are "primer dimers"? How do they affect the PCR product? d) Name the organism from which Taq polymerase was extracted. e) Give two applications of PCR? f) Differentiate between Reverse Transcriptase PCR and Real Time PCR g) Name one method for genome sequencing.	15 (4+2+2+2+2+2+1)	CO3
	Section D	(2x10=20 Marks)	
Q	Statement of question (each question carries 10 marks)		CO
1.	a) Describe the structural component of a bacteriophage with a labelled diagram? b) Discuss how does the infection cycle of lambda phage differs from that of M13 phages? c) With the help of a well labelled diagram, explain how cosmid is used to clone long DNA fragments.	2+3+5	CO2
2.	a) Describe the role of following reagents while running an SDS-PAGE (i) Ammonium persulphate (ii) TEMED (iii) SDS (iv) B-Mercaptoethanol (v) Coomassie Brilliant Blue b) Discuss the application of Recombinant DNA technology in production of following products of human therapeutic interest: (i) Insulin (ii) Vaccines	5+5	CO4