MANIPAL INSTITUTE OF TECHNOLOGY
MANIPAL
(A constituent unit of MAHE, Manipal)

DEPARTMENT OF BIOTECHNOLOGY

VI SEMESTER B.TECH. (BIOTECHNOLOGY)

END SEMESTER EXAMINATIONS, APRIL/MAY 2024

SUBJECT: Genetic Engineering [BIO 2223]

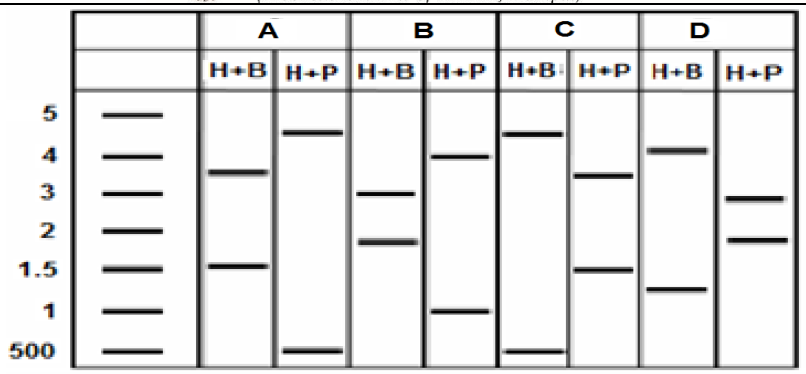
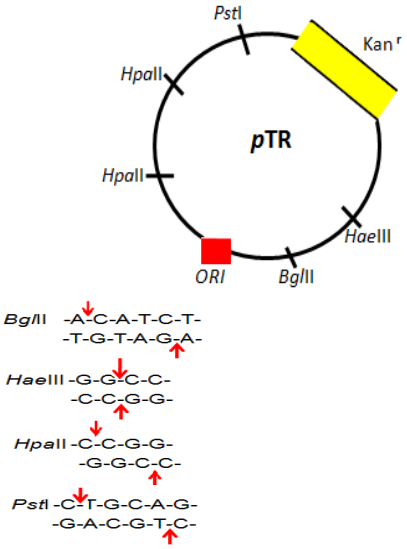
(Date: 09-05-2024 Time: 2:30 PM – 5:30 PM)

Time: 3 Hours **MAX. MARKS: 50**

Instructions to Candidates:

- Answer **ALL** the questions.
- Any data not provided may be suitably assumed.

Q. No	Question	M	CLO	AHEP4 LO	BL
1A	Explain why the promoter in general is rich in adenine (A) and thymine (T) bases?	2	1		2
1B	Why is biochemical purification of one gene away from the other genes in a cell more challenging than biochemical purification of one protein away from the other protein in a cell?	3	1		3
1C	How would you express the functional human P53 gene in a bacterial host? **Note:** The protein P53 is a polypeptide made up of chain A, B and C. During translation a single chain is produced and then undergoes a peptidolytic cleavage (post translational modification) to yield chain A, B & C and form a mature and functional protein.	5	1		4
2A	A gene of 2kb length into a vector of 3kb to make a GST fusion protein. The gene is being inserted at the *Eco*RI site and the insert has a *Hin*dIII site 500bp downstream of the first codon. You are screening for the clone with the correct orientation by restriction digestion of the plasmid using *Hin*dIII plus *Bam*HI (H+B) and *Hin*dIII plus *Pst*I (H+P). The map of the relevant region of the vector is shown below:	3	2		4

	 Given above is the pattern following restriction digestion of plasmid isolated from four independent clones (A, B, C or D). Which of the plasmids shown above represents the clone in the correct orientation?				
2B	A gene encoding for protein X was cloned in an expression vector under the T7 RNA polymerase promoter and *lac* operator. Cells were induced by the addition of 1 mM IPTG at 37□C for 6 h. Cells were lysed and fractionated into insoluble bodies and cell-free supernatant by centrifugation. Protein X is present in the insoluble bodies. Discuss the strategies would you use to express protein X in the cell-free supernatant?	**3**	**2**		**4**
2C	The plasmid *p*TR as a vector for incorporating foreign DNA into *E. coli* is used for cloning purpose. The plasmid map shows the locations of the restriction sites of four different restriction *endonucleases*, as well as the ORI and a kanamycin-resistance gene (kan^r) is given. The restriction cleaving sites for the enzymes are shown. Choose the best set of restriction enzymes from the table? Justify **Plasmid Map** **Recognition Sequences**  **First choice** / **Second choice** *Hpa*II / *Hpa*II *Bgl*II / *Bgl*II *Hae*III / *Hae*III *Pst*II / *Pst*II	**4**	**2**		**4**

<table>
<tr><td>3A</td><td>Describe the complete protocol by which the functional enzyme generates blunt ends from sticky ends in DNA fragments</td><td>5</td><td>3</td><td></td><td>4</td></tr>
<tr><td>3B</td><td>Is it possible to ligate two heterologous fragments cleaved with SseBI and StuI? Can the ligated DNA fragments be cleaved again with SseBI or StuI? Justify your answer.
Recognition site sequence of SseBI- AGG/CCT
Recognition site sequence of StuI- AGG/CCT</td><td>3</td><td>3</td><td></td><td>4</td></tr>
<tr><td>3C</td><td>List out the enzymes required for the following:
I. Removal of phosphate group
II. Cleavage of DNA
III. Addition of single stranded regions in double stranded DNA.
IV. Synthesizing cDNA and DNA for hybridization purposes</td><td>2</td><td>3</td><td></td><td>2</td></tr>
<tr><td>4A</td><td>Identify the technique and provide a brief overview of the reagents used to detect genome rearrangements such as translocations, deletions, and duplications as cancer progresses</td><td>2</td><td>4</td><td></td><td>3</td></tr>
<tr><td>4B</td><td>How does dideoxy nucleotide triphosphates method work for DNA sequencing?</td><td>5</td><td>4</td><td></td><td>2</td></tr>
<tr><td>4C</td><td>The λ bacteriophage genome (double stranded, linear DNA, 48,500 bp size) labeled at 5’ end with a radioactive phosphorous (32P) was digested with different restriction enzymes under conditions that permits partial digestion of the DNA. The resulting fragments were separated using agarose electrophoresis and bands were visualized by autoradiography. With the results shown in the below table, construct the λ phage restriction map.
<table>
<tr><th>Fragments</th><th>Apa I (bp)</th><th>Pvu I (bp)</th><th>Bam HI (bp)</th></tr>
<tr><td>1</td><td>48,500</td><td>48,500</td><td>48,500</td></tr>
<tr><td>2</td><td>10085</td><td>35,790</td><td>41,730</td></tr>
</table></td><td>3</td><td>4</td><td></td><td>4</td></tr>
</table>

	3		**26250**	**34500**				
	4		**11,930**	**27,920**				
	5			**22,345**				
	6			**5,505**				
5A	Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder caused by mutations in the gene encoding dystrophin, a protein involved in maintaining membrane integrity in muscle cells. The dystrophin gene spans roughly 2.5 Mb and is spliced to form a 14 kb mRNA transcript consisting of 79 exons. The DMD phenotype results from frame shift mutations that disrupt the reading frame of the dystrophin mRNA resulting in a truncated protein. Based on what you know of dystrophin, Design a therapeutic strategy for DMD?					**3**	**5**		**3**
5B	What protein combinations operate within plant cells when Agrobacterium Ti plasmid vectors are utilized to produce transgenic plants?					**4**	**5**		**2**
5C	A few years ago, an international consortium was formed to uncover the locations of genetic variation in the human genome. The consortium worked to identify variations within the human population. **i.** What percentage of genomic nucleotides do you expect two randomly chosen people to have in common? Mention the types of variation. **ii.** How can you explain the comparatively little variation between human individuals? Why is an understanding of genomic variation useful for the study human?					**3**	**5**		**4**