Reg. No.										

MANIPAL INSTITUTE OF TECHNOLOGY

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A Constituent Institution of Manipal University

IV SEMESTER B.TECH. (BIOTECHNOLOGY)

END SEMESTER EXAMINATIONS, APRIL 2017

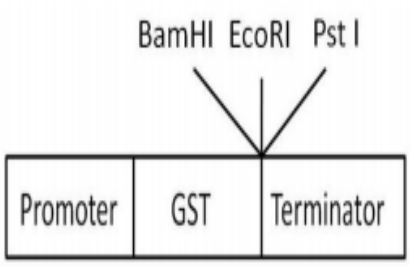
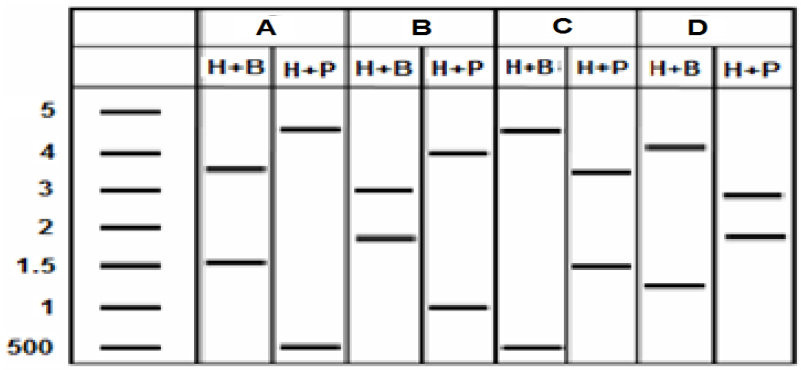
SUBJECT: GENETIC ENGINEERING [BIO 2203]

REVISED CREDIT SYSTEM
(21/04/2017)

Time: 3 Hours MAX. MARKS: 50

Instructions to Candidates:

- Answer **ALL** the questions.
- Missing data may be suitable assumed.

1A.	In both prokaryotes and viruses, a single mRNA can be translated into several different proteins. Which mechanisms explains this observation?	**3**
1B.	How would you express the functional human insulin gene in a bacterial host? **Note:** The hormone insulin is a polypeptide made up of chain A and B. During translation a single chain is produced and then undergoes a peptidolytic cleavage (post translational modification) to yield chain A & B, and form a mature and functional hormone.	**3**
1C.	If you add DNA to well at the top of gel. Should you place the positive electrode at the top or at bottom of the gel? Explain your choice.	**2**
1D.	Explain why the promoter in general is rich in adenine (A) and thymine (T) bases?	**2**
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:   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?	**2**

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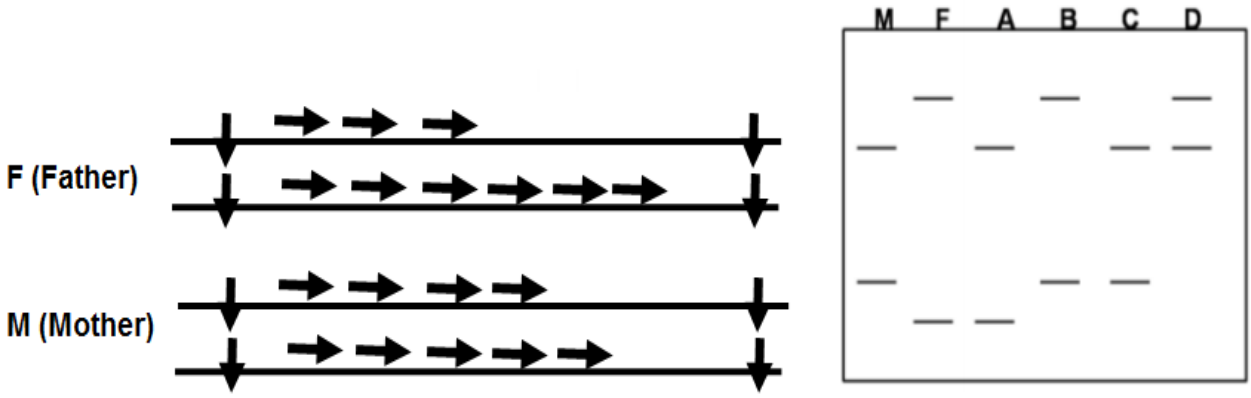
2B.	Comment with sketch on the following: **I.** Vectors that are useful for DNA sequencing. **II.** Vectors used for construction of genomic libraries.	**3**
2C.	A gene encoding for protein X was cloned in an expression vector under the T7 RNA polymerase promoter and *lac* operator. Cells were induced 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**
2D.	Outline the method that distinguishes self-ligated plasmid from recombinant plasmid. **Note:** Your answer should not be blue white/ insertional inactivation.	**2**
3A.	How does DNA *Ligase* work in joining two heterogeneous DNA fragments?	**3**
3B.	Is it possible to ligate two heterologous fragments cleaved with *Sse*BI and *Stu*I? Can the ligated DNA fragments be cleaved again with *Sse*BI or *Stu*I? Justify your answer. Recognition site sequence of *Sse*BI- AGG/CCT Recognition site sequence of *Stu*I- AGG/CCT	**3**
3C.	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	**2**
3D.	A new restriction enzyme was discovered in a microorganism from glaziers. Restriction enzyme has the recognition site of CAANNNTTG (where N is any base) and CCPyPyGG (where Py is any pyrimidine). Determine the average number of cuts the enzyme will produce.	**2**
4A.	As cancer progresses, several genome rearrangements including translocation, deletion, duplications etc. occur. If these rearrangements are to be identified, Brief the technique with reagent listing.	**3**
4B.	How does dideoxy nucleotide triphosphates method work for DNA sequencing?	**3**
4C.	A circular DNA plasmid was digested with the *Bam*HI, *Eco*RI and *Bam*HI+*Eco*RI. The results of AGE for *Bam*HI 6kb+4kb, *Eco*RI 6kb+4kb and *Bam*HI+*Eco*RI 1kb+4kb. Work out the restriction map of the plasmid.	**2**
4D.	Given that HIV integrates itself into the genome and that its sequence is known, what other method could be used to detect the presence of the HIV virus? Briefly describe this technique, listing the reagents that are necessary to perform it. (Hint: The amount of viral DNA in one human cell is not very great, how would you make more viral DNA).	**2**
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	**3**

	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?	
5B.	Agrobacterium Ti plasmid vectors are used to generate transgenic plants. What are the protein combinations which functions inside the plant cells?	**2**
5C.	Minisatellites are used as marker for identifying individuals via DNA fingerprinting as the alleles may differ in the number of repeats. From the Southern blot shown below identify the progeny (A, B, C and D) for the given parents (M= mother, F= father). Explain. 	**2**
5D.	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 chosenpeople to have in common? Mention the types of variation. **II.** How can you explain the comparatively little variation between human individuals? **III.** Why is an understanding of genomic variation useful for the study human?	**3**