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Manipal Institute of Technology, Manipal

(A Constituent Institute of Manipal University)

VII SEMESTER B.TECH (INDUSTRIAL BIOTECHNOLOGY)

END SEMESTER EXAMINATIONS, NOV/DEC 2015

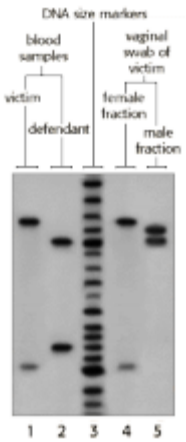
SUBJECT: GENOMICS & PROTEOMICS [BIO 431]

REVISED CREDIT SYSTEM

Time: 3 Hours MAX. MARKS: 50

Instructions to Candidates:

- Answer **ANY FIVE FULL** the questions.

1A.	The strength of a particular bond is measured by the amount of energy released upon the formation of the bond. The stronger the bond, the more energy is released. The change in energy (DG) that accompanies bond formation is used to describe the strength of a bond. The value of *G* is calculated according to the following equation: DG = −*RT* ln Keq. Where *R* is the universal gas constant (8.314 J K−1 mol−1*)*, *T* is the absolute temperature and ln *K*eq is the natural log of the equilibrium constant between the bonded and the unbonded forms of the molecule. Comment on DG values for covalent bond and hydrogen bonds in a DNA molecule.	***(4)***
1B.	Prabhu claims that he has achieved the sequencing of 166 Kbp T4 bacterio-phage genome in a single stretch by using automated sequencer. Akash doubted the result and ordered a thorough probe on Prabhu's claim. Why Prabhu's result was denied by Akash?	***(3)***
1C.	The key portion of the autoradiograph from a single locus probe analysis of various DNA samples in a rape investigation is shown in this figure: (3)  Samples of DNA were loaded into the following lanes: 1. Known blood sample of victim 2. Known blood sample from defendant 3. DNA size markers 4. Female fraction from vaginal swab of victim 5. Male fraction from vaginal swab of victim.	***(3)***

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	If you are the DNA analyst. What should you conclude?	
2A.	A 16 kb linear DNA is cut with EcoRI and BamHI. The EcoRI digest yields fragments of 2, 6, and 8 kb. The BamHI digest yields fragments of 5 and 11 kb. An EcoRI BamHI double digest yields 2, 3, 5, and 6 kb fragments. If the 3 kb fragment from the double digest is used as the probe in a Southern blot of the EcoRI digest, to which fragment(s) will it hybridize?	***(3)***
2B.	Genomic DNA is isolated from bacteria. After a long digestion with a 6-base cutting enzyme that recognizes the sequence GAATTC, the DNA is not cut. The genome is 4 Mb, so there should be approximately 1,000 sites in the DNA. What is the most likely explanation?	***(3)***
2C.	All humans have the same set of genes, and the sequence of our base pairs is remarkably similar. However, this doesn't mean that we all have exactly the same nucleotide sequence in our genome. If this were the case,then all humans would be clones having exactly the same genetic code. The passage of the genetic code from generation to generation via the sperm and ova of our ancestors requires replication of DNA, and while replication is remarkably precise, errors occasionally occur and produce changes in the base sequence. Based on this statement, explain the difference between mutation and single nucleotide polymorphism.	***(4)***
3A.	Sushmita is interested in isolating DNA from white blood cell for her Leukemia research. Before amplication of the isolated DNA, she needs to cut it however she detests using restriction enzyme. Explain the PCR technique where she can use without restriction digestion.	***(4)***
3B.	Can the proteins in denatured state in SDS-PAGE be renatured? Give proper reasoning.	***(3)***
3C.	If the following mix of molecules were purified using size exclusion chromatography, what would be the order in which the molecules pass through the opening in the bottom of the column? Mixture containing: hemoglobin, 65,000 Daltons; myoglobin, 17,000 daltons; myosin, 180,000 daltons.	***(3)***
4A.	Priya has isolated spindle pole body protein Spc42 (less abundant protein) from the yeast proteome. Initially, she has utilized SDS PAGE to characterize Spc42 protein and failed in it. Why SDS PAGE didn't work for her? Can you resolve this problem and help her to characterize the protein.	***(2)***
4B.	If the genome is not available in public database. Which sequencing technique can be performed?	***(2)***
4C.	Proteins "work together" by actually binding to form multicomponent complexes that carry out specific functions. These functional units can be as simple as dimeric transcription-factor complexes or as complex as the 30-plus component systems that form ribosomes. Biochemists have come to appreciate that essentially all proteins bind to or interact with at least one other protein. The discovery that proteins in higher organisms (e.g., human and mouse) contain higher numbers of functional domains	***(6)***

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	suggests that many of these proteins have multiple associations. How these protein complexes work is essential to understand how cells work as systems. Draw a flow chart in step-wise fashion to explain the work-flow?	
5A.	Generally bait-reverse bait approach has been used for studying protein and nucleotide interaction. Can we use the same approach for protein network mapping? Justify your answer by developing a protocol and explain it.	***(2)***
5B.	Affinity chromatography and SELDI-TOF can both use antibodies as the "capture" molecule to selectively sort out a target protein. How do these techniques differ from each other and how can each use antibodies? Which of these two techniques can identify normal from Alzheimer's β-amyloid protein and why is this the case? Each technique requires that the targeted protein is released from the capture antibody. How does each differ in this way?	***(6)***
5C.	What is the common way to isolate multi-protein complexes? Mention the complications during this experiment?	***(2)***
6A.	In protein microarrays, surface chemistry plays a crucial role for high throughput ana lysis.Detail the protein immobilization step using a work flow. Your experimental ingredients are your desired protein, glass, glutaraldehyde, APTES ((3-Aminopropyl) triethoxysilane.	***(6)***
6B.	Can we use the above mentioned surface chemistry for PDMS microarray? (PDMS: Poly dimethyl siloxane)	***(1)***
6C.	Post-translational modifications affect protein function. How can we detect Post-translation modifications of proteins? Write the assays used to figure out post-translational modified protein.	***(3)***