Reg. No.										

Manipal Institute of Technology, Manipal

(A Constituent Institute of Manipal University)

IV SEMESTER B.TECH (BIOTECHNOLOGY)

END SEMESTER EXAMINATION, MAY 2016

SUBJECT: GENETIC ENGINEERING [BIO 2203]

REVISED CREDIT SYSTEM

Time: 3 Hours MAX. MARKS: 50

Instructions to Candidates:

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

1A.	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?	**2**
1B.	Explain why the Ori site in general is rich in adenine (A) and thymine (T) bases?	**2**
1C.	Why are bacterial cells preferred for amplification of eukaryotic gene but NOT for expression of eukaryotic Gene?	**2**
1D.	The process in rDNA technology: a) Cut the cell's DNA with a restriction enzyme b) Cut many copies of a vector that has the flucloxacillin resistance gene with the same restriction enzyme c) Ligate each of the human DNA fragments into a cut vector d) Add DNA ligase to form a bond between the human DNA fragments and the vectors e) Transform the pool of recombinant vectors (vectors that contain a human DNA fragment) into bacterial cells and f) Select cells that have acquired a plasmid. Explain the nature of bacterial cells in step e and step f with the given example.	**2**
1E.	When foreign DNA and plasmid are both cut with the same restriction enzyme and mixed together, will all molecules form recombinants? Justify.	**2**
2A.	Design a vector, considering the following requirements: **i.** Should replicate in *E.coli* **ii.** Should have functional section markers **iii.** Should replicate in yeast **iv.** Should express the desired protein in a host cell **v.** Should be inexpensive	**4**
2B.	Explain at length the temperature controlled λ phage molecular cloning system.	**4**
2C.	Discuss the advantages of using cell free expression system.	**2**

<table>
<tr><td>3A.</td><td>Is it possible to ligate two heterologous fragments cleaved with Bam HI and Bgl II? Can the ligated DNA fragments be cleaved again with Bam HI or Bgl II?
Recognition site sequence of Bgl II AIGATCT
Recognition site sequence of Bam HI GIGATCC</td><td>3</td></tr>
<tr><td>3B.</td><td>If a DNA polymerase contains a mutation that allows it to strongly bind RNA strands, can the polymerase now function as an RNA polymerase? Why or why not?</td><td>3</td></tr>
<tr><td>3C.</td><td>Discuss the DNA ligase mechanism of action with illustration.</td><td>4</td></tr>
<tr><td>4A.</td><td>Compare and contrast chemical and enzymatic sequencing techniques.</td><td>2</td></tr>
<tr><td>4B.</td><td>Which parameter would you change first, if your PCR reaction gives too many products? What would you do if the PCR reaction gives very little, if any, of the correct product?</td><td>3</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>
<tr><td>3</td><td></td><td>26250</td><td>34500</td></tr>
<tr><td>4</td><td></td><td>11,930</td><td>27,920</td></tr>
<tr><td>5</td><td></td><td></td><td>22,345</td></tr>
<tr><td>6</td><td></td><td></td><td>5,505</td></tr>
</table></td><td>2</td></tr>
<tr><td>4D.</td><td>Why does adding NaCl to a DNA solution increase the Tm of a DNA duplex? How does increasing the NaCl concentration affect the "specificity" of a hybridization reaction?</td><td>3</td></tr>
<tr><td>5A.</td><td>Results from a single locus VNTR probe DNA fingerprint analysis for a female and her five children are given below. Identify the lane contains the DNA of the mother? Explain. AGE DNA fragment size: Lane 1- 2kb, 3kb Lane 2-2kb, 5kb Lane 3-1kb, 4kb Lane 4-2kb, 4kb Lane 5-4kb Lane 6-2kb, 5kb.</td><td>3</td></tr>
<tr><td>5B.</td><td>Why is the ex-vivo gene therapy more successful than the in- vivo gene therapy? Give three reasons.</td><td>3</td></tr>
<tr><td>5C.</td><td>Explain, how a plant tissue culture technique is made use of, in raising transgenic plants?</td><td>4</td></tr>
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