B.TECH/BT/5TH SEM/BIOT 3103/2016

RECOMBINANT DNA TECHNOLOGY
(BIOT 3103)

Time Allotted : 3 hrs **Full Marks : 70**

Figures out of the right margin indicate full marks.

Candidates are required to answer Group A and any 5 (five) from Group B to E, taking at least one from each group.

Candidates are required to give answer in their own words as far as practicable.

Group – A
(Multiple Choice Type Questions)

1. Choose the correct alternative for the following: **10 × 1 = 10**

(i) Which sequence is a palindrome?
(a) 5'ACGATTCG3' (b) 5'CCATT3'
(c) 5'AGGCCT3' (d) 5'CCAGG3'.

(ii) The enzyme used for 5' end labeling of DNA is
(a) Klenow fragment (b) DNA pol-I
(c) Polynucleotide kinase (d) Terminal transferase.

(iii) To be a cloning vector a plasmid need not have
(a) high copy number (b) a restriction site
(c) an antibiotic resistant marker (d) origin of replication.

(iv) What is the correct order of three basic steps of conventional PCR?
(a) Denature, anneal, & strand displacement
(b) Denature, extension, anneal
(c) Strand displacement, synthesis & release
(d) Anneal, denature, extension.

(v) Why are gene libraries constructed?
(a) To find new gene
(b) To sequence whole genome
(c) To create a "bank" of the genes in an organism
(d) All of the above.

(vi) Restriction endonuclease generated DNA fragments separated by gel electrophoresis and blot transferred onto a membrane filter are probed with a radioactive DNA fragment. This procedure is called:
(a) Gene cloning (b) The Southern technique
(c) The polymerase chain reaction (d) Recombinant DNA.

(vii) Two plasmids are said compatible when
(a) they can co exist in the same bacterial cell
(b) carry the same antibiotic gene
(c) carry the same toxin gene
(d) all of the above.

(viii) If you start with 2 copies of ds DNA molecules, and perform six cycles of standard PCR, how many double stranded copies of the starting material will you get?
(a) 32 (b) 128 (c) 64 (d) 16.

(ix) A scientist spread bacteria on a nutrient agar plate and after some time transferred the resulting bacterial colonies to three other plates (replica plating) such that the relative positions of the bacterial colonies were the same in all four plates. The fresh plates all contained the same antibiotic. The scientist notes that on each of the antibiotic-containing plates, a few colonies survive the antibiotic. Which of the following is most likely to be observed?
(a) The number of resistant colonies varies from plate to plate
(b) The number of resistant colonies is about the same in all three
(c) Both the number and position of resistant colonies are the same on all three plates
(d) Insufficient information is provided to make a prediction.

(x) Which one of the following is a goal of the Human Genome Project?
(a) Obtain the DNA sequence of the entire human genome
(b) Analyze the genomes of model organisms
(c) Develop programs focused on understanding and addressing the ethical, legal and social implications of the results obtained from the Human Genome Project
(d) All of the above are goals of the Human Genome Project.

Group – B

2. (a) What do you understand by the terms iso schizomers, neoschizomers, iso caudomers? Give examples of each highlighting the advantages of each in gene cloning.

(b) Suppose we have isolated the gene of interest by cleavage with Bam HI, and we have cleaved the vector with Bgl II, can we ligate the two without the linkers or adaptors? If yes, can the hybrid site (Bam HI/Bgl II) be recleaved with any of the enzymes? Explain.

9 + 3 = 12

3. (a) Define plasmid. Why it is not considered as genome?

   (b) Enlist the characteristics required for plasmids to be used as ideal cloning vector with labelled diagram. Give example.

   (c) Discuss the advantages and disadvantages of lambda phage as a vector over pBR 322.

(1 + 2) + 6 + 3 = 12

Group – C

4. (a) Describe the method of end labelling of DNA probe by biotin with diagram. Describe the chromogenic detection of the biotin labelled DNA probe with diagram.

   (b) What is the difference between normal PCR and QPCR? Explain the mechanism of the real-time PCR with TaqMan^{R} probe with diagram.

   (c) An aliquot of template DNA containing 3 X 10^4 copies of target gene is placed into PCR reaction. The reaction has a mean efficiency of 85%. How many cycles are required to produce 2×10^{10}.

(2 + 3) + (1 + 4) + 2 = 12

5. (a) What is restriction mapping? Find out the restriction map of pUC plasmid from digested fragments given (all are in kb units): BamHI:17; EcoRI:17; HindIII:10, 7; BamH1/EcoRI1:12, 5; BamH1/HindIII:10, 6, 1; EcoRI/HindIII:7, 6, 4.

   (b) Describe Sanger's method of DNA sequencing with labeled diagram and write the advantages & disadvantages of it.

   (c) A researcher desires to clone a gene (1 kb) of a microorganism. Its genome size is 1.5x 104 kb. The average size of its library fragment is 5 kb. The genomic library was created in vectors that were transformed into bacterial cells. If there is a 95% probability of the transformation, how many recombinant bacterial colonies will have to be screened to find this particular gene?

(1 + 3) + (3 + 2) + 3 = 12

Group – D

6. (a) What is cDNA library?

   (b) Describe preparation of cDNA library by schematic diagram. What are the advantages of cDNA library over a genomic DNA library?

   (c) Explain one screening technique for detection of recombinant cell.

2 + (4 + 3) + 3 = 12

7. (a) What is gene library? Schematically explain the construction of a genomic DNA library.

   (b) Why gDNA library of a prokaryotic organism is often constructed by partial digestion of two different enzymes?

(2 + 6) + 4 = 12

Group – E

8. (a) What is DNA vaccine? Describe the method of production of DNA vaccine against an antigen, with labelled diagram.

   (b) What are the different applications of genetic engineering? Describe the method of development of an insect resistant plant.

(1 + 4) + (3 + 4) = 12

9. (a) Describe with a flow chart, the cloning of human interferon gene to produce recombinant human interferon in a prokaryotic host.

   (b) Describe two detection methods for identification of pathogenic virus or bacteria.

   (c) Describe the steps to cure SCID by gene therapy.

4 + (2 + 2) + 4 = 12