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 of the following enzymes is not required for cloning a DNA fragment?
(a) Reverse transcriptase (b) DNA polymerase
(c) DNA ligase (d) Peptidyl transferase.

(ii) Which of the following components terminates the chain in a Sanger's sequencing reaction?
(a) Deoxynucleotides (b) Taq polymerase
(c) Oligonucleotide primer (d) Dideoxynucleotide.

(iii) What type of chemical bond formation is catalyzed by DNA ligase?
(a) Glycosidic (b) Hydrogen
(c) Phosphoester (d) Ester.

(iv) BAC can accommodate approximately:
(a) 5 to 10 kb of insert (b) 50 to 100 kb of insert
(c) less than 45 kb of insert (d) more than 100 kb of insert.

(v) In which application fluorescent antibodies are used?
(a) Immunohistochemistry (b) Flowcytometry
(c) Fluorescence activated cell shorting (d) All of the above.

(vi) A researcher desires to clone a gene of a microorganism. Its genome size is 1.5×10^4 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?
(a) 7000 (b) 8000 (c) 9000 (d) 10000.

(vii) A contig is
(a) a map of genetic markers that separated by less than 1cM
(b) a map showing the order of cloned bits of DNA
(c) unique DNA sequences that serve as molecular markers
(d) sets of two or more partially overlapping cloned DNA fragments.

(viii) You are subcloning a fragment of genomic DNA into an *E. coli* plasmid vector. As a first step, you cut out the fragment from an eXisting clone using a restriction enzyme. You then ligate the fragment into a similarly-digested plasmid vector carrying an amp^r gene. The site you ligate into is in the middle of the *lacZ* gene coding for β-galactosidase. After ligation, you transform *E. coli* with the ligated molecules using a $CaCl_2$ solution or electroporation and plate on X-gal plates with ampicillin. Successful transformation is indicated by _____, while successful insertion of DNA into the vector restriction site is indicated by _____.
(a) amp^r colonies; white colonies (b) white colonies; amp^r colonies
(c) amp^r colonies; blue colonies (d) no colonies; white colonies.

(ix) You want to express human insulin protein in *E. coli.* Given that you already have a clone containing the pig insulin gene, place the following steps in the proper order:
A. probe cDNA library with pig insulin gene clone
B. isolate mRNA from human pancreas
C. express human insulin in culture
D. using reverse transcriptase, make cDNA
E. grow up positive clones that hybridize to pig gene
F. clone cDNAs into expression vector to make library
(a) AECBDF (b) FABDEC (c) ABDFEC (d) BDFAEC.

(x) You are trying to restriction-map a plasmid. An EcoRI digest gives you a 10 kb fragment, an XhoI digest gives you a 2 kb and an 8 kb fragment, and the double digest gives you fragments of 2, 3, and 5 kb. From these results, you deduce that the size of the plasmid is _____ kb, the number of EcoRI sites is _____, the number of XhoI sites is _____, and that there is an EcoRI site within the _____ kb XhoI fragment.
(a) 10; 2; 1; 8 (b) 10; 1; 2; 8
(c) 20; 2; 2; 8 (d) 20; 1; 2; 8

Group – B

2. Discuss the detailed reaction mechanism, source and application of the following enzymes in recombinant DNA technology:
(i) DNA ligase,
(ii) Reverse transcriptase,

(iii) Terminal deoxynucleotide transferase,
(iv) Polynucleotide kinase.

(3 × 4) = 12

3. (a) How pBR322 was designed?

(b) Discuss the cloning strategy at the Bam HI site of pBR322.

(c) Discuss the advantages and disadvantages of lambda phage as a vector over pBR322.

3 + 6 + 3 = 12

Group – C

4. (a) What is restriction mapping?

(b) Three restriction endonucleases (RE-X, RE-Y and RE-Z) are used to cut a piece of linear DNA, singly and in pair wise combination. Sizes of fragments (in kb) are listed in order of size, *not* in linear order. Determine the correct order of restriction sites, and draw the map, with the intervals between sites labeled. X) 11, 6, 5; Y) 14, 8 Z) 16,6; and X x Y) 8, 6, 5, 3; X x Z) 11, 5, 5, 1; Y x Z) 8, 8, 6.

(c) Describe the technique only by labeled diagram, how you will purify eukaryotic mRNA.

(d) With a labeled reaction diagram, describe how you will make a 3'-end labeled DNA probe.

1 + 5 + 3 + 3 = 12

5. (a) Describe a method of preparation of random, 5'-end and 3'-end labelled radioactive probe only by label diagram.

(b) PCR is typically used to amplify DNA that lies between two known sequences. Then how you will amplify the end sequences of DNA whose internal sequences is known to you?

(c) A cloned DNA was sequenced with a method of DNA sequencing without electrophoresis but using bioluminescence. Describe that technique of DNA sequencing by labelled diagram.

(d) The restriction endonuclease EcoRI recognizes the sequence GAATTC. If a 40.96 kb genomic DNA with random sequence digested with EcoRI, theoretically how many fragments will be produced? (Presume that 50% GC content in the genomic DNA).

3 + 3 + 4 + 2 = 12

Group – D

6. (a) Describe the technique, only by labelled diagram, how you will purify eukaryotic mRNA. What is its importance?

(b) Explain blue-white screening for detection of recombinant cells.

(6 + 2) + 4 = 12

7. (a) Describe cloning of a DNA fragment produced by PCR without the use of restriction enzyme and DNA ligase, with labelled diagram.

(b) Describe the mechanism of the control of overexpression of a cloned gene in a pET vector system with label diagram.

(c) Describe the method of construction of a cDNA libraries for eukaryotic organism with a label diagram.

4 + 5 + 3 = 12

Group – E

8. (a) The rarest mRNA in a cell of a particular type has a concentration of five molecules per cell. Each cell contains 450,000 mRNA molecules. A cDNA library is made from mRNA isolated from this tissue. How many clones will need to be screened to have a 99% probability of finding at least one recombinant containing a cDNA copy of rarest mRNA?

(b) The restriction endonuclease EcoRI recognizes the sequence GAATTC. If a 40.96 kb genomic DNA with random sequence digested with EcoRI, theoretically how many fragments will be produced? (presume that 50% GC content in the genomic DNA.

(c) Describe the molecular mechanism of RNA interference and its use.

(d) What are the basic features of ribozyme and how are they used in gene therapy?

3 + 2 + 4 + 3 = 12

9. (a) Describe a flow chart for cloning of human growth hormone(hGH) gene to produce recombinant hGH.

(b) Describe two detection methods to identify pathogenic virus.

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

4 + 4 + 4 =12