ADVANCED GENETIC ENGINEERING
(BIOT 5101)

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) To clone a 100-300 kb DNA, which vector will be the best?
(a) Plasmid (b) Cosmid
(c) PAC (d) Lambda based vector.

(ii) An mRNA coding for a secretory protein, when translated using free ribosome under *in vitro* conditions, resulted in a 40 kDa protein. The same mRNA when translated using the rough endoplasmic reticulum resulted in a 36 kDa protein. The difference in the molecular weight of the two polypeptides is due to the loss of a
(a) 2 kDa peptide from N-terminus and a 2 kDa peptide from the C-terminus
(b) 1 kDa peptide from N-terminus and a 3 kDa peptide from the C-terminus
(c) 4 kDa peptide from the N-terminus
(d) 4 kDa peptide from the C-terminus.

(iii) Restriction endonuclease generated DNA fragments separated by gel electrophoresis and blot transfered 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.

(iv) YAC behaves similar to normal chromosomes because it possess
(a) centromere (b) centromere and telomere
(c) telomere and AR (d) centromere, telomere and ARS.

(v) 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.

(vi) Which of the following is an application of PCR?
(a) site directed mutagenesis
(b) amplification of specific segments of DNA
(c) for cloning into vectors
(d) all of the above.

(vii) Single stranded T-DNA from Ti plasmid in *Agrobacterium* is produced by the action of
(a) *chvA* and *chvB* (b) *vir* D1 and *vir* D2
(c) *vir* E1 and *vir* E2 (d) *vir* B1-B11.

(viii) What organism was the first to have its entire genome sequenced?
(a) Escherichia coli (b) Haemophilus influenza
(c) Vibrio cholera (c) Homo sapiens.

(ix) 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.

(x) *Luc, gfp, β-gluc* are genes well known as
(a) visible marker gene (b) reporter gene
(c) selectable marker gene (d) transgene.

Group – B

2. (a) The restriction enzyme HindIII recognizes sequence "AAGCTT". If the genomic DNA of random sequence of size 100 kb is cleaved with HindIII, what will be the average size of the fragments produced and how many fragments will be there?

(b) Write short notes on *any two (*with diagram)
(i) Lambda gt11 (ii) pBluescriptKSII+/- (iii) Shuttle vector.

(c) Write about the application of linker and adapter in genetic engineering.
3 + (3 + 3) + 3 = 12

3. (a) What is primer? What are the parameters one must take into account when designing the primer for PCR amplification?

(b) PCR 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) You are trying to restriction-map a plasmid. An EcoRI digest gives you a 10 kb fragment, and XhoI digest gives you a 2 kb and an 8 kb fragment, and the double digest by EcoRI and XhoI gives you fragments of 2, 3, and 5 kb. From these results, you deduce restriction map of the plasmid.

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

Group – C

4. (a) Write short notes about ***any two*** of the following:
   (i) Southern Blotting
   (ii) Radio labeling of DNA at 5' end, 3' end and internal base
   (iii) Pyrosequencing of DNA.

   (b) There is a protein G in a eukaryotic system, whose sequence of amino acid is known, describe the steps to clone the gene of protein G using a pUC18 vector.

   (c) A researcher desires to clone a gene (1 kb) of a microorganism. Its genome size is 1.5×10^4kb. 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, then how many recombinant bacterial colonies will have to be screened to find this particular gene?

(3 × 2) + 4 + 2 = 12

5. (a) Describe DNA cloning methods by using restriction enzyme, PCR, and ligation, with labelled diagram.

   (b) Describe the steps of making cDNA library of eukaryotic cell with labelled diagram.

   (c) 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?

4 + 5 + 3 = 12

Group – D

6. (a) Describe the process of developing transgenic mice with a flow chart.

   (b) Compare the advantages and disadvantages of the following methods for raising both transgenic plants and animals:
   (i) PEG-mediated protoplast fusion
   (ii) Microinjection.

6 + (3 + 3) = 12

7. (a) Describe in detail Particle bombardment technique.

   (b) Compare and contrast the advantages and disadvantages of Agro-mediated gene delivery with the above method.

   (c) Write short note on: chloroplast transformation.

6 + 3 + 3 = 12

Group – E

8. Write short notes on the following:
   (i) Strategies for genome sequencing,
   (ii) Large scale analysis of gene expression at RNA level.
   (iii) Human gene therapy.

(4 × 3) = 12

9. Write short notes on:
   (i) Yeast two hybrid system
   (ii) Production of recombinant protein in *E.coli*.

6 + 6 = 12