M.TECH/BT/1ST SEM/BIOT 5101/2016

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) 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.

(ii) 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.

(iii) A temperature of 75^0C will terminate DNA synthesis by *E.coli* DNA polymerase I. This is because
(a) *E.coli* DNA polymerase I is denatured at this temperature.
(b) the DNA is denatured at this temperature.
(c) the primers are denatured at this temperature.
(d) the temperature is too high for enzymatic reactions to occur.

(iv) Which of the following terms describes the situation when gene regulation occurs by short dsRNA molecules triggering an enzymatic reaction that degrades the mRNA of a target gene?
(a) Post Transcriptional Gene Silencing (b) RNAi
(c) co-suppression (d) all of the above.

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

(vi) One physical method for gene transfer technique for animal is
(a) Ca-phosphate mediated gene delivery
(b) Tri-Parental mating
(c) Electroporation
(d) Liposome-mediated.

(vii) A vector (e.g. plasmid) constructed in such a way that it can replicate in at least two different host species, allowing a DNA segment to be tested in several cellular settings, is called
(a) shuttle vector (b) recombinant plasmid
(c) transgene (d) none of these.

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

(ix) Pure plasmid DNA was isolated from a bacterium. Restriction enzyme digestion of this plasmid with either *Bam* HI or *Eco* RI resulted in two DNA fragments. A double digestion of the same plasmid with both these enzymes resulted in three DNA fragments. From this we can conclude that the isolated plasmid DNA is
(a) double stranded and linear (b) double stranded and circular
(c) single stranded and linear (d) single stranded and circular.

(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 is cleaved with HindIII, what will be the average size fragments produced?

(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 + 4 = 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.

(b) What are the disadvantages of expression of a eukaryotic protein in a prokaryotic host?

(c) Describe the mechanism of over expression is controlled in pET vector.

(2 + 2) + 3 + 5 = 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) What are ES-cells? How are they produced? Describe the transfection method to ES cells.

(b) Describe the process of developing transgenic sheep Dolly in detail.

(3 + 3) + 6 = 12

7. (a) Define and give example of: shuttle vector.

(b) What are Ti and Ri-plasmids? Where are they found?

(c) Mention the functions of all *vir*-genes in the natural process of gene delivery of *Agrobacterium* to plant cells.

3 + (2 + 2) + 5 = 12

Group – E

8. Write short notes about the following: **3 × 4 = 12**
(i) Strategies for genome sequencing,
(ii) Large scale analysis of gene expression at RNA level.
(iii) Human gene therapy

9. Write about any three of the following: **3 × 4 = 12**
(i) RNAi technology
(ii) AFLP
(iii) DNA micro array
(iv) Yeast two hybrid.