M.TECH/BT/1ST SEM/BIOT 5101/2014
2014

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 x 1=10

(i) Choose the right combination of components required to set up a polymerase chain reaction from the following
(a) Template DNA, two primers, dNTPs and DNA ligase
(b) Template DNA, two primers, NTPs and DNA ligase
(c) Template RNA, two primers, NTPs and DNA polymerase
(d) Template DNA, two primers, dNTPs and DNA polymerase

(ii) 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**.**

(iii) 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

(iv) A 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

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(v) Which of the following can be used for transferring the DNA into the host cells?
P. Transformation Q. Sonication R. Transfection S. Electroporation
(a) Only P can be used (b) Only Q & R can be used
(c) Only Q, R & S can be used (d) Only P, R & S can be used

(vi) Match the techniques mentioned in Column A with their applications given in Column B.

A	B
P. PCR	1. Identification of transcription factor binding sites in chromatin
Q. DNA microarray	2. Identification of HIV infected patients using serum samples
R. ELISA	3. Isolation of mouse homologue of a yeast gene
	4. Analysis of differential gene expression in cancer and normal cells

(a) P-4, Q-1, R-3 (b) P-3, Q-4, R-2
(c) P-4, Q-1, R-2 (d) P-3, Q-2, R-1

(vii) Protein binding regions of DNA are identified by one of the following techniques
(a) finger printing (b) southern blotting
(c) foot printing (d) western blotting

(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 IPTG and 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) 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

(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

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2.(a) Describe the pyrosequencing methods of DNA with labelled diagrams only. Why these methods called pyrosequencing?

(b) What are the differences between Snager dideoxy methods and pyrosequencing methods?

(c) Based on which important enzymatic reaction these above methods are developed? Write that reaction.

(d) Starting with 600 template DNA molecules, after 25 cycles of PCR, how many molecules of DNA will be produced? (4+1)+ 3 +2+2 = 12

3.(a) What is vector and what is host?

(b) Describe the features of a good vector and a good host in recombinant DNA technology.

(c) Describe the technique with labelled diagram based on which you will purify eukaryotic mRNA.

(d) What is restriction mapping? Three restriction endonucleases (RE-X, RE-Y and RE-Z) are used to cut a piece of linear DNA, **single and in pairwise** 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 labelled. **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. 2+4+3+3 = 12

Group - C

4.(a) What are the problems of cloning DNA into a vector, when DNA and vector both will be digested with single Restriction enzyme? How these problems can be solved? Describe with diagram.

(b) Describe one selection technique for positive clones containing of insert DNA**.**

(c) Write the names of different techniques to clone DNA without the use restriction enzyme and DNA ligase. Describe any one technique by labelled diagram only. (2+2) +4+(1+3) = 12

5.(a) Describe the steps for the cloning of a gene X from prokaryotic organism into PUC18 with single restriction enzymes, with labelled diagram only.

(b) Why the efficiency of getting positive clone by above method will be low? What

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are the different ways you can improve the cloning efficiency of this method? Describe 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+(2+3)+3 = 12

Group - D

6.(a) What are ES-cells? How are they produced?

(b) Describe the transfection method to ES cells.

(c) Describe the process of developing transgenic mice in detail. (3+2)+2+5 = 12

7.(a) What is T-DNA?

(b) Describe its structure in different strains of *Agrobacterium*.

(c) Mention the steps of generating a transgenic plant by **any one** of the following methods: (i) Gene-gun method. (ii) Agro-mediated gene transfer.

(d) Compare the advantages and disadvantages of these two methods**.** 2+1+6+3 = 12

Group - E

8.(a) Describe the molecular mechanism of RNA interference.

(b) What is the difference between antisense RNA technology and RNAi technology?

(c) What is biopharmaceutical? Describe the cloning of tPA by labelled diagram. 4+4+(1+3) =12

9. Write Short notes about the following: (4 X 3) = 12
(a) Human gene therapy,
(b) Human Genome project,
(c) DNA finger printing forensic science.