Name : .....

Roll No. : .....

Invigilator's Signature : .....

CS/M.Tech (BT)/SEM-2/MBT-201/2010

2010

GENETIC ENGINEERING

Time Allotted : 3 Hours Full Marks : 70

The figures in the margin indicate full marks.

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

GROUP – A

(Objective Type Questions)

Answer *all* of the following. 10 × 1 = 10

1. i) What is precisely protected in an RNAse Protection assay ?

   ii) A blood sample from an individual will produce a unique DNA fingerprint, also called a DNA profile. This is because each individual has a unique set of

   a) Proteins in his/he cells

   b) Mutations his/her DNA

   c) RNA in his/her nuclei

   d) Enzymes in his/her mitochondria

   e) Amino acids in his/her blood.

   iii) Name a technique to study DNA-protein interactions.

iv) Name two compounds used as cryoprotectants.

v) Ampicillin does not affect existing cells with intact cell envelopes but kills dividing cells as they synthesize new peptidoglycan. Is the statement *True* or *False* ?

vi) A chromatographic column in which oligo-dT is linked to an inert substance which is useful in separating eukaryotic mRNA from other RNA molecules. On what principle does this column operate ?

vii) The DNA shown below is from the 3' end of the β-*globin* gene, which is mutated in sickle cell anemia (autosomal recessive). Which bands would be seen in a Southern blot of DNA from normal subjects digested with *Eco*RI and hybridized with Probe *A* ?

Fig.

a) 6 kb band only

b) 6 kb band and 10 kb band

c) 4 kb band, 6 kb band, and 10 kb band

d) 4 kb band and 10 kb band

e) 4 kb band only.

viii) A particular RFLP is diagrammed below. 'E' represents invariant *Eco*RI restriction sites. '*' represents polymorphic *Eco*RI sites. The dark box represents the location of a particular DNA probe '*A*'. What are all the possible alleles (*i.e.* size of bands) seen on a Southern blot probed with '*A*' ?

Fig.

a) 1 kb, 2 kb, 3 kb, 4 kb, 5 kb, 6 kb

b) 1 kb, 3 kb, 4 kb, 6 kb

c) 3 kb, 4 kb, 6 kb

d) 2 kb, 3 kb, 6 kb

e) None of these.

ix) Choosing from the list below, which is a reasonable sequence of steps for cloning a piece of foreign DNA into a plasmid vector ?

I. Transform competent cells

II. Select for the lack of antibiotic resistance gene #1 function

III. Select for the plasmid antibiotic resistance gene #2 function

IV. Digest vector and foreign DNA with *Eco*RI, which inactivates antibiotic resistance gene # 1.

V. Ligate the digested DNA together.

a) IV, V, I, III, II

b) IV, V, I, II, III

c) I, III, IV, II, V

d) III, II, I, IV, V

e) None of these.

x) Many biotechnology compaines work with recombinant DNA plasmids with the goal of producing large quantities of a particular human protein. Once a human gene has been recombined with a bacterial plasmid, what must be done to get the human protein produced ?

a) Cut the recombinant plasmid with restriction enzymes

b) Put the recombinant plasmid into bacterial cells

c) Sequence the recombinant gene

d) Separate the human DNA from the plasmid DNA using electrophoresis

e) Make many copies of the gene using PCR.

GROUP – B

Answer *all* of the following questions. $6 \times 10 = 60$

2. a) Describe very briefly what is the alpha complementation test.

b) Illustrate the phenomena of restriction-modification (R-M) of phage λ on *E. coli C* and *E. coli K*.

c) Describe the ability of lambda ZAP to serve as an insertion vector for the construction of *c*DNA expression libraries. $2\frac{1}{2} + 2\frac{1}{2} + 5$

OR

a) How would you utilize selectable marker genes and markers for screening in DNA cloning experiments ?

b) Explain the mode of selection of recombinants using the Spi phenotype.

c) What does the acronym YAC stand for ? What special advantages do YACs offer in genomic analysis ? 4 + 3 + 3

3. a) Explain schematically the cloning of genomic fragments in any one high capacity vector that you know of.

b) Many gene products (proteins) when overexpressed may be toxic to the host cell. Explain with a suitable example how the inducibility of toxic gene products is dealt by the host cells.

c) Name two appropriate vector systems used for expressing proteins fused to protein tags. 4 + 4 + 2

OR

a) Merely increasing annealing temperature may not be sufficient to reduce non-specific amplification of undesired products during PCR. Suggest any suitable means (with proper examples) to conduct PCR in order to reduce non-specific product amplification.

b) What is RNAase protection assay and why is it preferred over conventional northern blotting experiments ?

c) In Real time PCR, which of the two methods, Sybr green or Taqman probe, is more reliable in monitoring DNA synthesis/product formation and why ? $2\frac{1}{2} + 2\frac{1}{2} + 5$

4. a) Some cancers are caused by the overexpression of a "normal" protein. What therapeutic strategy can be used to treat this type of disease ?

   b) Viral-based vectors may be utilized to correct the defect in patient's cells. Briefly explain a method which seems practical.

   c) How would you achieve site-directed mutagenesis using PCR techniques ?

   d) What are "doped" oligos and how are they helpful in generating mutant plasmids ? $2\frac{1}{2} + 2\frac{1}{2} + 2\frac{1}{2} + 2\frac{1}{2}$

OR

   a) Distinguish between primer walking and chromosome walking ?

   b) What are reporter genes and how are they useful for promoter analysis ? Explain with a suitable example.

   c) How would you select cells that are stably transfected ?

   d) Name any one method for introducing foreign gene into mammalian cells. 3 + 3 + 3 + 1

5. a) What are heterologous expression systems and what are their main uses ?

   b) Single-stranded DNA is used as a template in one of the methods in site-directed mutagenesis. What is that method ?

   c) In an expression construct, the junction between the promoter and coding sequence can be made in two functionally different ways, that is, transcriptional or translational fusions. Describe what is meant by a transcriptional fusion and a translational fusion. What are their relative advantages and disadvantages ?

   d) How can microarray be used for comparative studies ? 2 + 1 + 2 + 5

OR

You are asked to develop a laboratory model (for example a rat model) for a certain human live disease that results from excessive alcohol consumption, and subsequently study the gene expression profile. However, you have human liver arrays to carry out the experiments. You are also interested to test the effect of a new drug that could be a potential medication for such a disease.

a) Describe how you would set up the laboratory model.

b) With the help of flow diagrams explain how you would study the gene expression profiles using microarray technology. Remember that you also have to use this technology to see if the drug is effective or not.

c) How would the rat model help you to study human genes ?

d) What parameters would help you to normalize your data ?

e) Name the different technologies that have emerged for the fabrication of microarrays.

f) Name the dyes used for labelling probes in microarrays.

1 + 4 + 1 + 1 + 1 + 2

6. a) What is a microstatellite ? Give a very brief description.

b) What type of DNA methods are RAPD and RFLP ?

c) Describe briefly how RAPDs differ from more standard applications of this type of method.

d) Describe the technique of DNA footprinting in brief.

e) What are VNTRs ? Explain how their analysis is useful in DNA fingerprinting. 2 + 3 + 1 + 2 + 2

OR

By using the techniques of genetic engineering, scientists are able to modify genetic materials so that a particular gene of interest from one cell can be incorporated into a different cell.

a) Describe a procedure by which this can be done.

b) Explain the purpose of each step of your procedure.

c) Describe how you could determine whether the gene was successfully incorporated.

d) Describe an example of how gene transfer and incorporation have been used in biomedical or commercial applications. $2\frac{1}{2} + 2\frac{1}{2} + 2\frac{1}{2} + 2\frac{1}{2}$

7. You have attempted to ligate a 1·8 kb fragment foreign DNA into the *Eco*RI site in the multiple cloning site of the 0·4 kb plasmid vector shown below :

Fig.

a) After ligation you use the DNA in the ligaton mixture to transform host bacteria. Why is it important to use host bacteria that are deficient for restriction modification ?

b) You screen the bacteria that supposedly have been transformed with recombinant plasmid DNA. Some of the bacterial colonies growing on the nutrient agar plate that contains ampicillin and *X*-gal are white and some are blue. Explain these results.

c) To confirm the presence of the foreign DNA insert, you perform *Eco*RI restriction endonuclease digests on DNA extracted from bacterial colonies. Draw a diagram of an agarose gel showing the orientation of the positive and negative electrodes and the pattern of bands (label their size in kilobases) you would expect to see for *E*coRI-digested recombinant plasmid and *Eco*RI-digested non-recombinant plasmid vector, after electrophoresis and staining of the gel with ethidium bromide.

$2\frac{1}{2} + 2\frac{1}{2} + 5$

OR

Fig.

a) The nucleotide sequence of a DNA fragment was determined by the Sanger (dideoxy) DNA sequencing method. The data are shown above. What is the 5' → 3' sequence of the nucleotides in the original DNA fragment ?

b) Explain why ddNTPs are called "chain terminators" in DNA sequencing reactions.

c) What are the disadvantages in Maxam-Gilbert method of sequencing ?

d) Distinguish between adaptors and linkers.

e) Dot/Slot blotting or Northern blotting are both techniques to quantify transcripts. However, they differ in some aspects. Explain in brief on what aspects they differ. 2 + 2 + 2 + 2 + 2