Name : .....

Roll No. : .....

Invigilator's Signature : .....

CS/M.TECH(BT)/SEM-1/MBT-101/2011-12

2011

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
(Multiple Choice Type Questions)

1. Choose the correct alternatives for any *ten* of the following :

$10 \times 1 = 10$

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) The DNA restriction site recognized by the enzyme *Hind* III is – *TTCGAAAAGCTT*. Which of the following sequence contained in a double stranded DNA is cut by *Hind* III ?

ATTCATGC ATGCGA (S) *CTTATGC ATGCAAG (R)*

CGAAATGC ATGCTT (Q) *AAGATGC ATGCCTT (P)*

a) Only *Q* b) Both *Q* and *R*

c) Only *R* d) All of these.

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 *C*-terminus

b) 1 kDa peptide from *N*-terminus and a 3 kDa peptide from *C*-terminus

c) 4 kDa peptide from *N*-terminus

d) 4 kDa peptide from *C*-terminus.

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*.

	Column-*A*		**Column-*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 which one of the following techniques ?

a) Finger printing b) Southern blotting

c) Foot printing d) Western blotting.

viii) Si RNA(s) interfere at

a) transcriptional level

b) DNA replication level

c) post-transcriptional level

d) translational level.

ix) The 50 mL of competent *E.coli* cells (10^9 CFU/mL) were transformed using 0·5 ng of a 5 kb plasmid DNA to which 950 mL of SOC medium was added. Only 50 uL of this was plated on a selective agar plate. After an 12h incubation at 37°C, 90 colonies were observed. Calculation of the efficiency of this transformation in CFU/lug of DNA is

a) $3 \cdot 6 \times 10^5$ b) $3 \cdot 6 \times 10^6$

c) $1 \cdot 8 \times 10^5$ d) $1 \cdot 8 \times 10^6$.

x) A researcher desires to clone a gene (1 kb) of a microorganism. Its genome size is 1·5 × I 04 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.

xi) Transgenic mice are produced by

a) *in vitro* fertilization of ova by sperms from a different strain followed by implantation

b) transfer of cloned foreign DNA into blastocyst cells followed by implantation

c) implantation of mixed blastocyst cells from two different strains

d) selection of a given trait by repeated back-crossing.

xii) A plasmid can be transformed into *Agrobacterium* by

a) $CaCl_2$-phenol mediated gene delivery

b) Tri-Parental mating

c) Electroporation

d) all of these.

xiii) One *in vivo* gene delivery system to animal cells is

a) Microinjection b) Liposome

c) Vector d) Amplicon.

xiv) Chromosomal genes necessary for T-DNA transformation are

a) *chv A* and *chv B* b) *vir*-operon

c) LB and RB d) none of these.

GROUP – B

(Short Answer Type Questions)

Answer any *three* of the following $3 \times 5 = 15$

2. Write short note on any *one* of the following :

a) pBR322

b) pUC18

c) Cosmid

d) Directional cloning.

3. a) Is it possible to ligate a DNA fragment claved with *Bam*HI to a heterologous fragment that had been cleaved with the restriction enzyme *Bgl* II ? Can the ligated DNA fragments be cleaved again with both *Bam*HI and *Bgl* II ? Explain.

   b) Why is formaldehyde used to denature RNA in a Northern blot, isn't RNA single stranded to begin with ? Why can't NaOH be used to denature RNA in the Northern blotting procedure as it is in the Southern Blotting procedure ? 2 + 3

4. Write only the reaction and one use of the following enzymes in genetic engineering : 5 × 1

   i) Klenow fragment

   ii) T_4 DNA ligase

   iii) Bacterial alkaline phosphates

   iv) Polynucleotide kinase

   v) Terminal deoxynucleotide tranferase.

5. a) What is nick translation ? Describe with diagram.

   b) Describe the features of a good vector and a good host in genetic engineering. 2 + 3

6. a) The restriction enzyme *Hind*III recognizes sequence "AAGCTT". If the genomic DNA of random sequence, size 50 kb is cleaved with *Hind*III, what will be the average size of a fragments and how many fragments will be there ?

   b) A stock of human genomic DNA has a concentration of 275 µg/ml. You wish to cut 5 µg DNA with 10 units of restriction enzyme *Hind*III. The *Hind*III enzyme has a concentration of 2500 units/ml. What volumes of DNA and enzymes should be used ? 2½ + 2½

7. a) Mention three different methods of gene transfer to animal cells.

   b) Mention two selectable markers and two reporter genes for plant gene delivery system.

   c) Name one shuttle vector and one co-integrate vector.

   2 + 2 + 1

8. Describe in detail the gene transfer technique used for the generation of transgenic sheep Dolly.

GROUP – C

(Long Answer Type Questions)

Answer any *three* of the following. 3 × 15 = 45

9. a) Write requirement of PCR.

   b) Describe the detailed mechanism of PCR (draw a flow diagram)

   c) How will you optimize the PCR reaction condition ?

   d) Write the applications of PCR in biology.

   e) What is RT-PCR ? How will you synthesize ds cDNA ?

   2 + 3 + 3 + 3 + 4

10. a) Write in details about the features of a general expression vector with a diagram.

    b) Write the mechanism of the control of expression of a cloned gene in a pET vector system with diagram. 8 + 7

11. Write short notes on any *three* of the following : 3 × 5

    a) Colony hybridization

    b) Maxum-Gilbert DNA sequencing methods

    c) Eukaryotic mRNA isolation

    d) South-Western blotting

    e) Inverse PCR

    f) Reverse PCR.

12. a) Describe the Snager dideoxy methods of DNA sequencing with diagram.

    b) Describe the pyrosequencing methods of DNA with diagrams. Why this methods called pyrosequencing ?

    c) What are the differences of Snager dideoxy methods and pyrosequencing methods ?

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

5 + (4 + 1) + 3 + 2

13. a) What is restriction enzyme (RE) ? Write the names of different types of RE.

    b) Compare properties of the different types of restriction enzymes.

    c) Which types of RE's are useful for genetic engineering and why ? 2 + 5 + 3 + 5

14. Write about the Application of genetic engineering in any *three* of the following : 3 × 5

    a) Human gene therapy

    b) Fighting against AIDS

    c) DNA based diagnosis of genetic diseases

    d) Recombinant protein hormone.

15. a) What is T-DNA ? Describe its structure in different strains of *Agrobacterium*.

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

c) What are the advantages of Agro-mediated gene delivery ? 2 + 5 + 5 + 3

OR

a) What is the importance of embryogenic stem cells ?

b) How are they produced ? Describe the transfection method to ES cells.

c) Describe the process of developing transgenic mice in detail. 3 + 2 + 2 + 8

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