Name : .....

Roll No. : .....

Invigilator's Signature : .....

CS/M.Tech(BT)/SEM-1/MBT-101/2012-13

2012

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

   ii) A *m*RNA coding for a secretory protein, when translated using free ribosome under *in vitro* conditions, resulted in a 40 kDa protein. The same *m*RNA when translated using the rough endoplasmic reticulum resulted in a 36 kDa protein. The difference in the molecular weights 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) A temperature of 75°C 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 describe when gene regulation occurs by short *ds*RNA molecules triggering an enzymatic reaction that degrades the *m*RNA of a target gene ?
   a) Post Transcriptional Gene Silencing
   b) RNAi
   c) Co-suppression
   d) All of these.

v) Which of the following statements about DNA isolation from *E.coli* is not correct ?
   a) Chemical extraction using phenol removes proteins from DNA
   b) RNA is removed from the sample by RNase treatment
   c) Detergent is used to break apart plant cells to extract DNA
   d) Lysozyme digests peptidoglycan in the bacterial cell wall.

vi) Which of the following terms describe when gene regulation occurs by short *ds*RNA molecules triggering an enzymatic reaction that degrades the *m*RNA of a target gene ?
   a) Post-transcriptional Gene Silencing
   b) RNAi
   c) Co-suppression
   d) All of these.

vii) How are restriction enzyme (RE) and T4DNA ligase used in genetic engineering ?

a) Restriction enzyme cuts the DNA at specific site, producing ends that can be ligated back together with ligase

b) Only restriction enzyme that produces blunt ends after cutting DNA can be ligated with ligase

c) Only restriction enzyme that produces sticky ends on the DNA can be ligated with ligase

d) Restriction enzyme randomly cuts DNA and the cut fragment can be ligated back together with ligase.

viii) Which of the following techniques would allow a researcher to determine the genetic relatedness between two samples of DNA ?

a) Inverse PCR

b) Reverse transcriptse PCR (RT-PCR)

c) Overlap PCR

d) Randomly amplified polymorphic DNA (RAPD).

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 these.

x) Why are gene libraries constructed ?

a) To find new gene

b) To sequence whole genome

c) To create a 'bank' of the genes in an organism

d) All of these.

xi) In genetic engineering, a novel DNA sequence formed by the combination of two non-homologous DNA molecules is

a) *c*DNA b) chimeric DNA

c) oligonucleotide d) none of these.

xii) 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, called

a) shuttle vector b) recombinant plasmid

c) transgene d) none of these.

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

a) Ca-phosphate mediate gene delivery

b) Tri-parental mating

c) Electroporation

d) Liposome-mediated.

xiv) *aad, ble, dhfr, npt* II, *aph* II are genes well known as

a) visible marker gene

b) reporter gene

c) selectable marker gene

d) transgene.

GROUP – B

(Short Answer Type Questions)

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

2. A genomic library of a prokaryotic organism is often constructed by cloning the products of a Sau3AI partial digest of the genomic DNA into a BamHI site of the vector.

a) Why are two different enzymes used in this experiment ?

b) What is a partial digestion and how is it performed ?

c) What is the significance of use of partial digestion during making of genomic libraries ? 1 + 2 + 2

3. 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) The human genome contains about 3×10^9 bp of DNA. How many 200 kb fragment would you have to clone into BAC library to have 90% probability of including a particular sequence ? 2½ + 2½

4. Write short note on any *one* with diagram :
   a) Lambda gt11
   b) pBluescriptKSII +/–
   c) Shuttle vector.

5. 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

6. Write short note on any *one* with diagram :
   a) cDNA synthesis
   b) DNA fingerprinting
   c) Biosafety measures of genetic engineering works.

7. Write only the reaction and *one* use of the following enzymes in genetic engineering : 5 × 1
   i) Taq Polymerase
   ii) E.coli DNA ligase
   iii) Reverse transcriptase
   iv) Polynucleotide kinase
   v) Terminal deoxynucleotide transferase.

8. Write 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) Describe the steps for the cloning of a gene *X* from prokaryotic organism into pUC18 with single restriction enzymes, with diagram only.

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

   c) What are the different ways you can improve the cloning efficiency of this method ? Describe with diagram.

   d) Describe the selection of positive clone by in pUC18 and the mechanism of selection. 3 + 3 + 5 + 4

10. a) Describe the mechanism of the control of expression of a cloned gene in a pET vector system with diagram.

    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 micro-organism. Its genome size is $1 \cdot 5 \times 10^4$ 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 ? 6 + 6 + 3

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

    a) Baculovirus vector

    b) PCR/OLA

    c) Radio labelling of DNA at 5′ end, 3′ end and internal base

    d) Pyrosequencing

    e) Touch down PCR.

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

    b) PCR is typically used to amplify DNA that lies between two known sequences. How will you amplify the end sequences of DNA whose internal sequences are known to you ?

    c) Describe different applications of PCR.

    d) An aliquot of template DNA containing 3×10^4 copies of target gene is placed into PCR reaction. The reaction has a mean efficiency of 85%. How many cycles are required to produce 2×10^{10} ? 3 + 4 + 3 + 5

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

    a) RNAi technology

    b) AFLP

    c) DNA micro-array

    d) Yeast two hybrid

    e) Human Genome Project.

14. a) What is the difference between normal PCR and QPCR ?

    b) Explain the real-time fluorescent PCR with TaqMan^R probe.

    c) How does this technique differ from real time PCR with SYBR^R Green ?

    d) In the process of *c*DNA library screening if you have ended up getting partial clones, how would you complete these partial clones with the help of PCR ?

    4 + 4 + 3 + 4

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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