RECOMBINANT DNA TECHNOLOGY
(BIOT 3103)

Time Allotted : 2½ hrs **Full Marks : 60**

Figures out of the right margin indicate full marks.

Candidates are required to answer Group A and any 4 (four) from Group B to E, taking one from each group.

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

Group – A

1. Answer any twelve: **12 × 1 = 12**

Choose the correct alternative for the following

(i) The following table provides information about four proteins. Which one of the following options correctly identifies the order of elution in size exclusion chromatography and the increasing order of mobility in SDS polyacrylamide gel?

Protein	Native mol. wt. (Da)	pI	Type
P	32000	6.4	monomer
Q	40000	8.5	homodimer
R	25000	4.9	monomer
S	45000	8.5	homotrimer

(a) Chromatography: PRQS; Electrophoresis: PRQS (b) Chromatography: SQPR; Electrophoresis: PRQS
(c) Chromatography: SQPR; Electrophoresis: RPQS (d) Chromatography: RPQS; Electrophoresis: SQPR.

(ii) In a typical gene cloning experiment, by mistake a researcher introduced the DNA of interest within ampicillin resistant gene instead of lac z gene. The competent cells were allowed to take up the plasmid and then plated in the media containing ampicillin, X-gal and IPTG and subjected to blue-white screening. Considering all plasmids were recombinant which one of the following statements correctly describes the outcome of the experiment?
(a) The bacteria which took up the plasmids would form white colonies
(b) The bacteria which took up the plasmids would not grow
(c) The bacteria which took up the plasmids would grow and give blue colonies
(d) All of the bacteria would grow and give white colonies.

(iii) Which of the following statement is not true for (SDS-PAGE)?
(a) Ethidium bromide is used to track the progress of electrophoretic mobility
(b) β-mercaptoethanol is used to reduce disulphide bonds
(c) The protein migrates towards the anode
(d) The lower molecular weight protein migrates faster than the larger molecular weight protein.

(iv) A gene was cloned into a unique *BamHI* restriction site present inside the ampicillin resistance gene of a vector that contains both ampicillin and tetracycline resistance genes. To select for only recombinant clones, the transformation mixture should be plated on which of the following plates?
(a) Ampicillin plus Tetracycline containing plate
(b) Ampicillin containing plate
(c) Tetracycline containing plate followed by replica-plating on ampicillin containing plate
(d) Ampicillin containing plate followed by replica-plating on Tetracycline containing plate.

(v) A hexacutter enzyme will cut a random DNA sequence once in every
(a) 256 bp (b) 4096 bp (c) 65,536 bp (d) all of (a), (b) & (c).

(vi) The *E. coli* strain must be used for bacterial expression vector system
(a) DH5 α (b) M13 (c) BL 21 (d) none of (a), (b) & (c).

(vii) The benefit of using qPCR is given below. Which statements are correct?
(P) It shows increased fluorescence after every cycle.
(Q) It shows a continuous increase in the fluorescence.
(R) It can be used to determine the number of transcripts in a given sample.
(S) It overcomes the inherent bias of the end point PCR
(a) (P) and (S) (b) (Q), (R) and (S) (c) (P), (Q) and (S) (d) (Q) and (R)

(viii) Given below are two sets of terms related to various methods used in recombinant DNA technology

Group-I	**Group-II**
(P) Streptavidin	(i) DNA-protein interaction
(Q) Southwestern blotting	(ii) FAM
(R) IMAC	(iii) Biotin
(S) TaqMan	(iv) Ni^{+2}

Which the correct match between group-I and Group-II

(a) P- (iv); Q - (iii); R - (i); S - (ii)
(b) P- (iii); Q - (ii); R - (iv); S - (ii)
(c) P- (i); Q - (ii); R - (iv); S - (iii)
(d) P- (iii); Q - (i) R - (iv); S - (ii).

(ix) Match the techniques mentioned in group-I with their applications given in group-II.

Group-I	Group-II
(P) PCR	(1) Identification of transcription factor binding sites in chromatin.
(Q)DNA microarray	(2) Identification of HIV infected patients.
(R) ELISA	(3) Identification of mouse homologue of a yeast gene.
	(4) Analysis of differential gene expression in cancer and normal cells.

Choose the combination of techniques that correctly list with their applications.

(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.

(x) Match between the application of genetic engineering (group-I) and nature or product related to the application (Group-II)

Group-I	**Group-II**
(P) *Ex-vivo* gene therapy	(1) Phyloene synthase
(Q) Golden rice	(2) Recombinant Human growth hormone
(R) Flavour saver tomato	(3) Antisense polygalctouronase
(S) Humatrope	(4) CFTR

Which one of the following is the correct match between group-I and group-II

(a) P- 4; Q–1; R–3; S–2
(b) P-1; Q–2; R–3; S–4
(c) P-2; Q–1; R–3; S–4
(d) P-1; Q–4; R–3; S–2.

Fill in the blanks with the correct word

(xi) *E.coli* bacteria of _____ phase culture should be used to make competent cells for transformation.

(xii) Based on the enzyme _____ reaction Sanger's method and pyrosequencing DNA sequencing techniques are developed.

(xiii) A relaxed plasmid is a _____ copy number plasmid.

(xiv) In pET vector system, _____ is used to control the expression of transgene.

(xv) T4 DNA ligase needs _____ as cofactor.

Group - B

2. (a) What do you understand by the terms isoschizomers, neoschizomers, isocaudomers? Give examples of each highlighting the advantages of each in gene cloning. *[(CO1)(Describe)/IOCQ)]*

(b) Suppose we have isolated the gene of interest (GOI) by cleavage with *BamHI*, and we have cleaved the vector (V) with *BglII*, can we ligate these two GOI and V without the linkers or adaptors? If yes, can the hybrid site (*BamHI*/*BglII*) be recleaved with any of these enzymes? Explain. *[(CO1)(Understand/IOCQ)]*

(c) What are linkers? Mention their different applications. *[(CO1)(Understand/LOCQ)]*

4 + 4 + 4 = 12

3. (a) Compare the following screening techniques for selection of positive clones:
Replica plating method and Blue-white selection. *[(CO4)(Discuss/IOCQ)]*

(b) Why host choice is of utmost importance in pUC –series of cloning vectors? *[(CO2) (Understand/HOCQ)]*

(c) Discuss the design of any one mammalian expression vector. *[(CO2)(Remember/LOCQ)]*

4 + 4 + 4 = 12

Group - C

4. (a) PCR is typically used to amplify DNA that lies between two known sequences, then how will you amplify the end sequences of DNA whose internal sequence is known? *[(CO3)(Understand/IOCQ)]*

(b) A cloned DNA was sequenced with a method of sequencing without PAGE but using one tube reaction with fluorescence. Explain the steps of the technique of DNA sequencing by labelled diagram. *[(CO2)(Understand/IOCQ)]*

(c) In a DNA ligation reaction, two different DNA solutions are supplied to you.
One is a 3.5 kb *SmaI* digested insert DNA with concentration of 1µg/10µl; and another is a 4.31 kb *SmaI* digested vector DNA with concentration 5µg/10µl. Calculate the amount of insert and vector DNA to be used for a ligation reaction by T4 DNA ligase (concentration 1U/µl), and also write the reaction protocol for a final volume of 40µl, with 10X ligase reaction buffer. *[(CO6)(Analyse)/HOCQ)]*

(d) The EcoRI recognizes the sequence GAATTC. If a 40.96 kb genomic DNA with random sequence digested with EcoRI, theoretically how many fragments will be produced? What will be the minimum size of the DNA fragments after digestion? (Presume that 50% GC content in the genomic DNA). *[(CO6)(Analyse)/IOCQ)]*

3 + 4 + (1 + 2) + (1 + 1) = 12

5. (a) What is the principle of separation of nucleic acids by agarose gel electrophoresis (AGE)? Why separation of intact genomic DNA not possible by standard AGE? Describe the modified principle of electrophoresis to separate intact genomic DNA. *[(CO2)(Analyse/LOCQ)]*

(b) Write the reaction mechanism for the polymerization of acrylamide to form polyacrylamide gel? *[(CO2)(Remember/IOCQ)]*

(c) A researcher desires to clone a gene (1 kb) of a microorganism. Its genome size is 1.5×10^4 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. *[(CO6)(Apply/HOCQ)]*

(2.5 + 1 + 2.5) + 3 + 3 = 12

Group - D

6. (a) Why cDNA library is prepared? Discuss the steps with a flow chart. *[(CO4)(Analyse/HOCQ)]*

(b) How can we get high level of gene expression by using pET vector system? *[(CO4)(Apply/IOCQ)]*

(4 + 6) + 2 = 12

7. (a) What are linkers and adapters? How they are needed in gene library preparation? *[(CO4)(Explain/IOCQ)]*

(b) Illustrate different methods for size fractionation of gDNA. *[(CO4) (Remember/LOCQ)]*

(c) How the efficiency of blunt-ended ligation can be increased? *[(CO4)(Apply/IOCQ)]*

4 + 4 + 4 = 12

Group - E

8. (a) Explain the general features of an expression vector, with a labelled diagram. *[(CO5)(Explain/IOCQ)]*

(b) Write the steps and explain the principle of 2nd generation DNA sequencing developed by 454 life sciences with a labelled diagram? *[(C54)(Remember/IOCQ)]*

(c) In a gene expression analysis experiment using qRT-PCR , for tumour cell and control cell sample, the following results was obtained.

	Gene	
Sample	C_T of p53 (target)	C_T of GAPDH (reference)
Control cell (calibrator)	14	15.5
Tumour cell (test)	10	16.5

Calculate the fold change in expression of p53 gene of tumour cell with respect to the control cell by Livack method. *[(CO6)(Analyse/HOCQ)]*

4.5 + 4.5 + 3 = 12

9. (a) Write the names of genome sequencing strategies used in HGP. Explain the steps of anyone of the genome sequencing strategies (that you have mentioned) with diagram. *[(CO5) (Understand)/IOCQ)]*

(b) Write the names of two single gene defective disease with the name of defective gene. Explain the steps of treatment of anyone of the single gene defective genetic disease you have mentioned by gene therapy. *[(CO5) (Explain/IOCQ)]*

(c) In a biotech company, someone wants to clone a gene-X (whose protein product can be used as biopharmaceutical) from a eukaryote. A cDNA library is made from mRNA isolated from this eukaryotic cell. The number of mRNA corresponding to gene-X has a concentration of 10 molecules per cell. Each cell contains total 40,000 mRNA molecules. How many clones need to be screened from the cDNA library to find at least one recombinant containing a cDNA copy of gene-X mRNA with 90% probability? *[(CO5) (Analyse)HOCQ)]*

(1 + 3) + (1 + 4) + 3 = 12

Cognition Level	LOCQ	IOCQ	HOCQ
Percentage distribution	18.75	54.16	27.08

Course Outcome (CO):

After the completion of the course students will be able to

CO1. Understand mechanism of action and the use of the different DNA modifying enzymes, vectors and host in recombinant DNA technology and solve and analyze the problems of restriction mapping.

CO2. Explain and demonstrate the different techniques of recombinant DNA technology like labelling of probe, DNA, RNA and protein sequencing, blotting and hybridization, microarray; ELISA; separate and identify nucleic acid and protein by electrophoresis and chromatography, and apply the knowledge to solve and analyse problem related to these techniques.

CO3. Demonstrate the mechanism of standard, quantitative and different modified polymerase chain reactions (PCR), use of PCR in DNA cloning and solve and analyse problems related to PCR.

CO4. Apply the different types of cloning and expression methods of gene in biotechnology and screen, identify, modify and analyse the cloned gene; explain the creation and screening of genomic and cDNA library in different vectors.

CO5: Understand and demonstrate the application of recombinant DNA technology in different fields of biotechnology like gene therapy, human genome project, production of recombinant vaccine, etc.
CO6: Analyze and solve problems related to rDNA technology.

**LOCQ: Lower Order Cognitive Question; IOCQ: Intermediate Order Cognitive Question; HOCQ: Higher Order Cognitive Question.*