ADVANCED GENETIC ENGINEERING
(BIOT 5101)

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) In a qRT-PCR experiment for quantitation of unknown RNA from a COVID19 patient sample, the $\mathbf{C_T}$ value was 12. What was amount of RNA copies present in the unknow sample?
(a) 4.096 copies (b) 2048 copies
(c) 8192 copies (d) 4096 copies

(ii) 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

(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

(iii) 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 ligated 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

(iv) Which of the following techniques is not involved in the identification of DNA at crime scenes against possible suspects?
(a) PCR (b) Western Blot
(c) DNA sequencing (d) DNA fingerprinting.

(v) Which can be transformed by gene gun?
(a) Any plant (b) Any animal
(c) Plant or animal (d) Microbes.

(vi) You are trying to restriction-map a plasmid. An EcoRI digest gives you a 10 kb fragment, and XhoI digest gives you a 2 kb and an 8 kb fragment, and the double digest gives you fragments of 2, 3, and 5 kb. From these results, you deduce that the size of the plasmid is _____ kb, the number of EcoRI sites is _____, the number of XhoI sites is _____, and that there is an EcoRI site within the _____ kb XhoI fragment?
(a) 10; 2; 1; 8; (b) 10; 1; 2; 8; (c) 20; 2; 2; 8; (d) 20; 1; 2; 8.

(vii) Which one of the following statements is correct?
(a) None of the *virulence* genes of *Agrobacterium tumefaciens* are expressed constitutively
(b) Integration of T-DNA with the nuclear genome of plant cells occurs only by homologous recombination
(c) Host plant genes do not play any role in *Agrobacterium-mediated* transfer of T-DNA into plant cells
(d) Opines are a source of nitrogen for *Agrobacterium* cells.

(viii) Different experimental approaches were used to quantify serum levels of IL-17 in human patient samples. Which one of the following approaches provides the most accurate quantification in a standard clinical setting?
(a) Sandwich ELISA with monoclonal capture and detection antibodies against the same epitope of human IL-17
(b) Fractionation of the serum sample on SDS-PAGE followed by Western blotting with polyclonal anti-human IL-17 antibody
(c) Direct ELISA by coating the plate with patient serum and detection with polyclonal anti-human IL-17 antibody
(d) Sandwich ELISA with monoclonal capture and detection antibodies against different epitopes of human IL-17

(ix) You want to express human insulin protein in E. coli. Given that you already have a clone containing the pig insulin gene, place the following steps in the proper order:

A. probe cDNA library with pig insulin gene clone
B. isolate mRNA from human pancreas
C. express human insulin in culture
D. using reverse transcriptase, make cDNA
E. grow up positive clones that hybridize to pig gene
F. clone cDNAs into expression vector to make library

(a) AECBDF; (b) FABDEC; (c) ABDFEC; (d) BDFAEC;

(x) An investigator identified a nuclear localization signal (NLS; Pro-Lys-Lys-Lys-Arg-Lys) at the C-terminus of the protein X (50 kDa). To analyze the localization of protein X the investigator fused protein X with GFP at the C-terminus. The fusion protein was detected in the cytosol. When the nuclear localization signal was fused with GFP at the N-terminus, the NLS-tagged GFP extensively localized in the nucleus. Based on this observation the investigator made a few hypotheses:

A. The basic amino-acid stretch in the protein X-GFP chimeric construct is masked by the GFP sequence and thus not capable of directing entry of protein X-GFP into the nucleus.

B. The X-protein in the full-length X-GFP chimeric protein is post-translationally modified that impacts its import into the nucleus.

C. Fusion with GFP makes the protein X too bulky to enter the nucleus through the nuclear pore complex?

(a) A and D (b) B only (c) A and B (d) C and D.

Fill in the blanks with the correct word

(xi) One suitable reporter gene for expression vector is _____.

(xii) _____ is a method for identifying the positions where individual DNA-binding proteins attach to a genome.

(xiii) The company developed the mRNA vaccine 1st in the world is _____.

(xiv) Name of an expression vector in prokaryotic host developed based on lac operon is _____.

(xv) To prevent cells from mechanical damage, media is supplemented with _____ during particle bombardment.

Group - B

2. (a) Describe a method of preparation of random, 5'-end and 3'-end labelled radioactive probe only by label diagram. *[(CO1)(Analyse/LOCQ)]*

(b) PCR typically used to amplify DNA that lies between two known sequences, then how you will amplify the end sequences of DNA whose internal sequences is known to you. *[(CO2)(Apply/IOCQ)]*

(c) A cloned DNA was sequenced with a method sequencing without the electrophoresis but using bioluminescence. Describe that technique of DNA sequencing by label diagram? *[(CO2)(Understand/LOCQ)]*

(d) The restriction endonuclease *EcoRI* recognizes the sequence GAATTC. If a 85 kb genomic DNA with random sequence digested with *EcoRI*, theoretically what will be the minimum size of a fragment and how many fragments will be produced? (Presume that 50% GC content in the genomic DNA). *[(CO6)(Understand/LOCQ)]*

3 + 3 + 4 + 2 = 12

3. (a) Write the mechanism of the reactions with the following enzymes in genetic engineering, with labelled diagram
(i) BP clonase, (ii) Klenow, (iii) *EcoRI* methylase, (iv) BAP, (v) TOPO-I. *[(CO1)(Understand/IOCQ)]*

(b) Explain the features of the following vectors with labelled diagram
(i) pBR4322, (ii) pUC18 *[(CO1)(Explain/IOCQ)]*

(c) If a 100 kb genomic DNA with random sequence is digested with *BamHI*, theoretically how many fragments will be produced? (Assume that 50% GC content in the genomic DNA). *[(CO6)(Analyse/HOCQ)]*

(5 × 1) + (2.5 × 2) + 2 = 12

Group - C

4. (a) Describe DNA cloning methods without using restriction enzyme and ligase with labelled diagram. *[(CO4)(Analyse/IOCQ)]*

(b) Describe the steps of making cDNA library of eukaryotic cell with labelled diagram? *[(CO4)(Remember/LOCQ)]*

(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. *[(CO6)(Apply/HOCQ)]*

4 + 5 + 3 = 12

5. (a) Describe the DNA cloning steps using YAC vector with diagram. *[(CO1)(Remember/LOCQ)]*

(b) What are the disadvantages of expression of a eukaryotic protein in a prokaryotic host? *[(CO3)(Analyse/IOCQ)]*

(c) Explain the general features of an expression vector with a diagram. Describe the mechanism of over expression in pET family vectors. *[(CO3)(Understand/HOCQ)]*

4 + 2 + (2 + 4) = 12

Group - D

6. (a) What are ES cells? Discuss the gene transfer technique to ES cells. *[(CO5)(Analyse/LOCQ)]*
   (b) List the advantages of the following gene transfer technique in specific cases Lipofection, Particle bombardment? *[(CO5)(Remember/LOCQ)]*

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

7. (a) How the microinjection technique is successfully used in raising transgenic mice? Elaborate the steps with a flow diagram. *[(CO5)(Analyse/HOCQ)]*
   (b) What is liposome? How DNA is incorporated in it? *[(CO5)(Remember/LOCQ)]*

(4 + 4) + (2 + 2) = 12

Group - E

8. (a) Explain the cloning of human TPA gene for the production of rh-tissue plasminogen activator, with labelled diagram. *[(CO2)(Remember/LOCQ)]*
   (b) Describe principle and steps of DNA finger printing to solve the parenting problem? *[(CO5)(Explain/IOCQ)]*
   (c) In a gene expression analysis experiment using qRT-PCR, for tumour cell and control cell sample, the following results were obtained.

Sample	Gene	
	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/IOCQ)]*

   (d) You have planned to make a genomic library using λEMBL3 vector, using Sau3A1 partial digest of the human genome (3 x 10^9 bp). If you wish a 99% chance of isolation of the desired gene from the λEMBL3 genomic library by screening of 68800 independent clones, then evaluate, what was the insert size of the library? *[(CO6)(Evaluate/HOCQ)]*

4 + 3 + 3 + 2 = 12

9. Write about principle, steps and application in genetic engineering of the following:
   (i) RNAi technology, (ii) mRNA vaccine, (iii) ELISA. *[(CO5)(Analyse/IOCQ)]*

(4 + 4 + 4) = 12

Cognition Level	LOCQ	IOCQ	HOCQ
Percentage distribution	39.58	38.54	21.88

Course Outcome (CO):

After the completion of the course students will be able to

CO1. Describe the function and application of the common enzymes used in molecular biology and explain the different DNA sequencing methods and when they would be applied.
CO2. Explain which biological hosts is the best choice for producing a certain protein and why.
CO3. Give examples of how to increase or decrease the expression of a given gene using gene regulation mechanisms.
CO4. Describe methods for performing DNA mutagenesis and how to screen or select for successful mutants.
CO5. Apply to produce of transgenic plants and animals and explain the principles behind modern gene therapy.
CO6. Apply the knowledge of genetic engineering in problem solving and in practice.

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