ADVANCED GENETIC ENGINEERING
(BIOT 5101)

Time Allotted : 3 hrs **Full Marks : 70**

Figures out of the right margin indicate full marks.

Candidates are required to answer Group A and any 5 (five) from Group B to E, taking at least one from each group.

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

Group – A
(Multiple Choice Type Questions)

1. Choose the correct alternative for the following: **10 × 1 = 10**

(i) Match the techniques mentioned in group A with their applications given in group B.

Group-A	Group-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

Which one of the following is the correct match between Group-A and Group-B?
(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.

(ii) A multimeric protein when run on SDS PAGE showed 2 bands at 20KDa and 40. However, when protein was run on native PAGE it showed single band at 120 KDa. The native form of protein would be
(a) homo trimer (b) hetero trimer
(c) hetero tetramer (d) hetero dimer.

(iii) In the following DNA sequence which have highest melting temperature?
(a) TGGGCCCTAATG (b) AAATTTATATATA
(c) GAGAGAGAGAGA (d) GCGCGCGCGCGC.

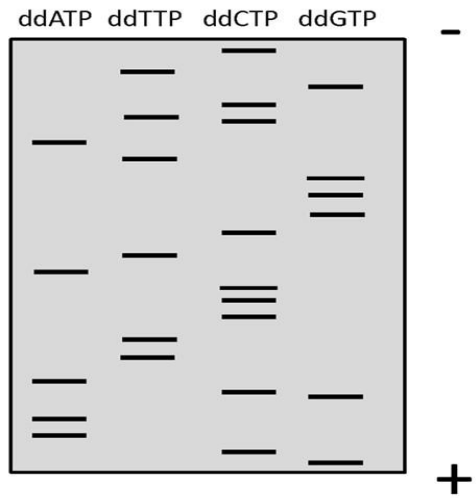
(iv) A scientist want to isolate 3 kb X gene from a genomic library of Mouse (genome size = 1.8 × 10^9 bp). He has screened 350,000 clones from the genomic library, what will be the probability to get the desirable gene fragment?
(a) 0.44 (b) 0.56 (c) 0.97 (d) 0.68.

(v) 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) FABDEC (b) AECBDF (c) BDFAEC (d) ABDFEC.

(vi) *E.coli* Bacteria of _____ phase can be used to make competent cells for transformation.
(a) Lag phase (b) Log phase,
(c) Stationary phase (d) Logarithmic decline phase

(vii) Shown here is a schematic of the image for a DNA sequencing gel using the Sanger's method. Based on your understanding of the methodology, identify the option that shows the DNA sequence of the template strand.

(a) 5'-CTGCTCATGGGCTACCCTTACGAACG-3'
(b) 5'-GACGAGTACCCGATGGGAATGCTTGC-3'
(c) 5'-GCAAGCATTTCCATCGGGTACTCGTC-3'
(d) 5'-CGTTCGTAAGGGTAGCCCATGAGCAG-3'.



(viii) A gene was cloned into a unique HindIII restriction site present in the ampicillin resistance gene of a vector that contains both ampicillin and kanamycin resistance genes. To select for only recombinant clones, the transformation mixture should be plated on which of the following plates?

(a) Ampicillin containing plate
(b) Ampicillin plus Kanamycin containing plate
(c) Ampicillin containing plate followed by replica-plating on kanamycin containing plate
(d) Kanamycin containing plate followed by replica-plating on ampicillin containing plate.

(ix) CaMV 35 S promoter is a/an

(a) Universal promoter (b) Systemic promoter
(c) Constitutive promoter (d) All of these.

(x) A mixture of monomeric proteins (W,X,Y,Z) eluted from Sephadex-G-200 column in the order W, X, Y, Z. The mixer of proteins was subjected to standard SDS-PAGE. What will be the order of their appearance of the proteins from top to bottom in SDS-PAGE?

(a) Z, Y, X, W (b) X, Y, Z, W (c) Y, Z, X, W (d) W, X, Y, Z.

Group – B

2. (a) Write the name of the of screening techniques for the clone at the level of DNA, RNA and protein (one for each)? Explain anyone techniques you have mentioned with diagram. [(CO1)(Understand/LOCQ)]

(b) Explain the principle and steps of real-time fluorescent PCR with TaqMan probe. [(CO1)(Understand/IOCQ)]

(c) An aliquot of template DNA containing 3×10^2 copies of target gene is placed into PCR reaction. The DNA is amplified of 19 cycles. The 10^8 copies of DNA produced at the end of PCR. Then, calculate what was the efficiency of thermal cycler? [(CO6)(Solve/HOCQ)]

(1 + 4) + 4 + 3 = 12

3. (a) Explain only the mechanism of the reactions of the following enzymes used in genetic engineering, with labelled diagram (i) BP clonase, (ii) *EcoRI* methylase, (iii) TOPO-I. [(CO1)(Understand/LOCQ)]

(b) Explain the special features of the following cloning vectors with labelled diagram:
(i) pJB8, (ii) pBeloBAC-11. [(CO1)(Explain/IOCQ)]

(c) You are trying to restriction-map a plasmid DNA. An *SalI* digest gives you a 12 kb fragment, and *EcoRI* digest gives you a 7 kb and an 6 kb fragment, and the double digest by *SalI* and *EcoRI* gives you fragments of 3, 4, and 6 kb. From these results, you deduce restriction map of the plasmid. [(CO6)(Analyse/HOCQ)]

(3 × 2) + (2 × 2) + 2 = 12

Group – C

4. (a) Write the names of two techniques of DNA cloning without the use of restriction enzyme and DNA ligase. Explain the steps of any one method that you mentioned above, with labelled diagram. [(CO2)(Analyse/IOCQ)]

   (b) There is a protein-X in a eukaryotic system, whose sequence of amino acid is known to you, explain principle and the steps to clone the gene of protein-X using a pUC18 vector with a labelled diagram only. How you will select the positive clone in pUC18 vector (explain the steps with labelled diagram)? [(CO3)(Explain/IOCQ)]

   (c) Calculate the amount of different component will be required for the standard restriction enzyme reaction with the following supplied samples and conditions (write in the tabular format). A DNA sample (concentration 1 µg/10 µl), RE enzyme *HindIII* and *SalI* (both 1U/µl). The final volume of reaction should be 60µl. You have to digest 2 µg DNA, with reaction 10X reaction buffer and 100XBSA. [(CO3)(Analyse/HOCQ)]

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

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

   (b) Someone cloned a DNA fragment in a plasmid as cloning vector using single RE and T4-DNA ligase and observed the efficiency of getting positive clone was very low (~25%). Explain why the efficiency of getting positive clone in this was low? How you can improve the cloning efficiency of this method? (Explain with labelled diagram). [(CO4)(Explain/HOCQ)]

   (c) In a transformation experiment, 0.05 ml of *E.coli* competent cells was added with 15 ng of ligated DNA (containing a recombinant plasmid with amp^R gene) in a tube. Then, it was kept in ice for 5 min. Then, heat shock was given at 42°C for 2 min. Then, 0.45 ml of LB media was added to it and then the tube was incubated for 30 min at 37°C temperature, in a shaker incubator before plating. Then, 0.1 ml transformed cells were spread on LB-agar plate containing ampicillin (100 ug/ml) and incubated at 37°C incubator for overnight. Next day, you observed colonies were present in the experimental plate and when you counted the colonies, you found that number colonies present in the plate was equal to ten times of the last two digits of your autonomy (exam) roll number. Now, you calculate the transformation efficiency of the experiment? [(CO6)(Evaluate/HOCQ)]

4 + (3 + 3) + 2 = 12

Group – D

6. (a) What is Ti-plasmid? Describe the structure of T-DNA. [(CO5)(Understand/LOCQ)]

   (b) Illustrate the role of *vir* genes in transfer of T-DNA. [(CO5)(Illustrate/IOCQ)]

(2 + 3) + 7 = 12

7. (a) What parameters are included in the construct for high level transgene expression in animal tissue? [(CO6)(Understand/HOCQ)]

   (b) Discuss the molecular mechanism of gene delivery by Ca3(PO4)3. [(CO6)(Remember/LOCQ)]

   (c) Discuss the microinjection technique used to transfer gene in animal cells. Why it is not applicable in plant cells? [(CO6)(Analyze/IOCQ)]

4 + 4 + 4 = 12

Group – E

8. (a) Write the names of three next generation sequencing (NGS) platform? Describe the principle and steps of anyone of NSG platform you have mentioned with labelled diagram. [(CO6)(Remember/LOCQ)]

(b) Mention the names of different types of vaccine according to their nature. Assume that you got a job in a biotech company and they asked you to prepare a plan for preparation of peptide vaccine against SARS-Cov2. Now you explain the logic and the steps of your plan to prepare the peptide vaccine with a diagram. [(CO6)(Apply/HOCQ)]

(c) Write three names genetic diseases. Explain, how you will do DNA based diagnosis of anyone of those genetic disease, the logic and the steps with diagram. [(CO6)(Explain/IOCQ)]

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

9. (a) Explain the strategy of Human gene therapy which was used for therapeutic treatment of Ashanti DeSilva with diagram. [(CO5)(Explain/HOCQ)]

(b) Explain the mechanism and steps of gene therapy to remove the defective gene by CRISPR/Cas9 tools. [(CO6)(Understand/IOCQ)]

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

Sample	Gene	
	C_T of p53 (target)	C_T of GAPDH (reference)
Control cell (calibrator)	18	14.5
Tumour cell (test)	12	15.5

Calculate the fold change in expression of p53 gene of tumour cell with respect to the control cell by Livack method (show all the steps of calculation).

[(CO6)(Analyse/IOCQ)] [(CO6)(Evaluate/HOCQ)]

4 + 4 + 4 = 12

Cognition Level	LOCQ	IOCQ	HOCQ
Percentage distribution	25	42.71	32.29

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.