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) 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) 20; 1; 2; 8 (b) 20; 2; 2; 8 (c) 10; 1; 2; 8 (d) 10; 2; 1; 8

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

(iii) Given below are a few statements related to enzymes and their functions in molecular reactions.
P. Alkaline phosphatases remove 3' phosphates from DNA and RNA.
Q. S1 nuclease removes single-stranded regions from partially double-stranded DNA.
R. 5' end-labelling of DNA molecules can be done by using polynucleotide kinase which transfers a ^{32}P-labelled phosphate group to the 5' end of dephosphorylated DNA.
S. 3'-5' exonuclease activity of Taq polymerase releases the reporter from the 3' end of TaqMan probes in qPCR
(a) P and S (b) Q and R (c) Q and S (d) P and R

(iv) Green fluorescence protein is suitable as
(a) Selectable marker gene (b) Visible marker gene
(c) Reporter gene (d) Antibiotic resistant gene.

(v) One physical method for gene transfer to animals is
(a) Ca-phosphate mediated (b) Sonication
(c) Electroporation (d) Liposome mediated.

(vi) A 160 kDa complex of four protein molecules, consists of a dimer formed by a 25 kDa protein connected by two disulfide bonds, and three other proteins of 10, 30 and 70 kDa, respectively. It was isolated and analysed on an SDS-PAGE gel without DTT in the gel loading dye. Which one of the following options would represent the SDS-PAGE profile?
(a) Four bands corresponding to 10, 30, 50 and 70 kDa
(b) Four bands corresponding to 10, 25, 30 and 70 kDa
(c) One band corresponding to 160 kDa
(d) Two bands corresponding to 50 kDa and 110 kDa.

(vii) 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 ligate 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) white colonies; amp^r colonies (b) no colonies; white colonies
(c) amp^r colonies; blue colonies (d) amp^r colonies; white colonies

(viii) A DNA sequence is given below: 5' - ATGACGA TGACGAGACGATGCAGA TGA TAGCAGTAGCGAA TGAC - 3' The following primers were designed to amplify the above sequence:
A. 5'-TACTGCT-3' B. 5'-CAGTAAG-3' C. 5'-ATGACGA -3'
D. 5'-GTCATTC-3' E. 5'-TCGTCAT -3' F. 5'-GAATGAC-3'
If we negate the effects of primer length, Tm, %GC and other factors, which one of the following options represents a combination of primers that could amplify the above DNA sequence
(a) A and C (b) B and D (c) E and F (d) C and D

(ix) A chimeric promoter is
(a) Promoters taken from different systems
(b) Promoter region taken from generally two different systems
(c) Promoter region taken and fused from generally two different systems
(d) CaMV35S promoter region upto -40.

(x) 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.

Fill in the blanks with the correct word

(xi) The enzyme used for 5' end labelling of DNA is _____.

(xii) TA cloning is developed based on a disadvantage property of an enzyme, is _____.

(xiii) In western blotting hybridization nature of the probe is _____.

(xiv) In SDS-PAGE protein visualizing chromogenic dye is _____.

(xv) In Agarose gel electrophoresis DNA mixture are separated based on _____.

Group - B

2. (a) Write only the mechanism of the reactions with the following enzymes in genetic engineering, with labelled diagram (i) LR 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 digested with *BamHI*, theoretically how many fragments will be produced? (Assume that 50% GC content in the genomic DNA). *[(CO6)(Apply/HOCQ)]*

(5 × 1) + (2.5 × 2) + 2 = 12

3. (a) Write three the differences between normal PCR and QPCR. *[(CO1)(Understand/IOCQ)]*

(b) Explain the real-time fluorescent PCR with TaqMan probe. *[(CO1)(Explain/IOCQ)]*

(c) In the process of cDNA library screening if you have ended up getting partial clones, how would you complete these partial clones into full length with the help of PCR? *[(CO2)(Apply/IOCQ)]*

(d) An aliquot of template DNA containing 3×10^2 copies of target gene is placed into PCR reaction. The efficiency of thermal cycler is 90%. Evaluate, how many cycles are required to produce 2×10^{10} copies of DNA? *[(CO6)(Solve/HOCQ)]*

3 + 3 + 4 + 2 = 12

Group - C

4. (a) Describe the DNA cloning steps using YAC vector with labelled 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

5. (a) Sourav is interested to clone a DNA fragment from a prokaryotic cell, whose sequence is known but during preparation of DNA fragment you are not getting suitable restriction enzyme (RE) site corresponding to the RE sites present in the MCS of pUC18 vector (by his choice). He completed the DNA cloning using PCR, RE and T4-DNA ligase. Now, you explain the steps of the cloning he followed. *[(CO3)(Explain/IOCQ)]*

(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)(Remember/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^{o}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.

[(CO2)(Apply/IOCQ)]

4 + (3 + 3) + 2 = 12

Group - D

6. (a) What are ES cells? Discuss the gene transfer techniques to ES cells. *[(CO5)(Analyse/HOCQ)]*

   (b) Illustrate the advantage of lipofection in specific cases. *[(CO5)(Remember/LOCQ)]*

   (c) Describe briefly the development of liposomes as gene delivery system. *[(CO2)(Apply/IOCQ)]*

(1 + 4) + 3 + 4 = 12

7. (a) Name the oncogenes present in *Agrobacterium*. Why they are not expressed in the bacteria but after infection are expressed in plants? How it affects the plants? *[(CO5)(Analyse/HOCQ)]*

   (b) Discuss the importance of CaMV35S promoter in plant expression vector. *[(CO4)(Remember/LOCQ)]*

   (c) Why that is replaced nowadays? *[(CO2)(Apply/IOCQ)]*

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

Group - E

8. (a) What is Next Generation Sequencing (NGS)? Describe any one technique of NGS with labelled diagram. *[(CO6)(Remember/LOCQ)]*

   (b) Explain the principle and steps of creation of superbug for purpose of bioremediation, with diagram. *[(CO6)(Understand/IOCQ)]*

   (c) Explain the mechanism of gene therapy to remove the defective gene by CRISPR/Cas9 tool. *[(CO6)(Analyse/HOCQ)]*

(1 + 3) + 4 + 4 = 12

9. (a) Write the names of three protein biopharmaceuticals. How you will clone anyone gene of those biopharmaceuticals? Explain the logic and steps with diagram. *[(CO6)(Explain/IOCQ)]*

   (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)(Apply/IOCQ)]*

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

Cognition Level	LOCQ	IOCQ	HOCQ
Percentage distribution	14.58	47.92	37.50