RECOMBINANT DNA TECHNOLOGY
(BIOT 3103)

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

(ii) With respect to sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE), which of this statement is not true?

(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.

(iii) 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) 4096 copies (b) 2048 copies (c) 8192 copies (d) 4096 copies.

(iv) In a ligation reaction between blunt end insert DNA (I) and blunt end vector DNA (V) by T4-DNA ligase, to get maximum efficient ligation product, the ratio of I:V will be

(a) 1:10 (b) 10:1 (c) 3:1 (d) 1:3.

(v) 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

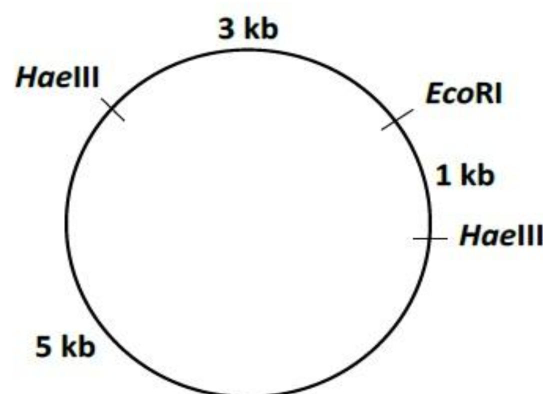
(a) P- (iv); Q - (iii); R - (i); S - (ii) (b) P- (iii); Q - (ii); R - (iv); S - (ii)
(c) P- (i); Q - (ii); R - (iv); S - (iii) (d) P- (iii); Q - (i) R - (iv); S - (ii)

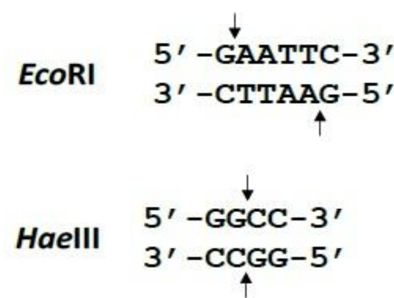
(vi) You want to express human insulin protein in *E. coli*. Given that you already have a clone containing the pig insulin gene, place the following steps in the proper order:

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.

(vii) The product of complete digestion of the plasmid shown below with *EcoRI* and *HaeIII* was purified and used as a template in a reaction containing Klenow fragment of DNA polymerase, dNTPs and [α-^{32}P]-dATP in a suitable reaction buffer. The product thus obtained was purified and subjected to gel electrophoresis followed by autoradiography. The number of bands that will appear on the X-ray film is ________.





(a) 4 (b) 3 (c) 2 (d) 1

(viii) Diagnosis of influenza virus infection can be done using some of the following techniques:

(P) Western blot and southern blot (Q) Northern blot and ELISA
(R) ELISA and RT-PCR (S) PCR and electron microscopy.

Choose the combination of techniques that correctly list the detection methods.

(a) P and R only (b) R and S only (c) Q and R only (d) P and S only.

(ix) CRISPR-CAS is a/an

(a) Artificial restriction enzyme (b) Type II restriction enzyme
(c) Type I restriction enzyme (d) None.

(x) To be a cloning vector a plasmid does not require

(a) high copy no (b) restriction site
(c) antibiotic selection (d) origin of replication.

Group–B

2. (a) Describe any one method for radiolabelling 5'end. How it is different from radiolabelling 3' end? [(CO2)(Remember/LOCQ)]

(b) How poly A-tailed RNA can be prepared *in vitro*? [(CO3)(Understand/HOCQ)]

(c) Compare the activities of different reverse transcriptase used in rDNA technology. [(CO4)(Analyse/IOCQ)]

(3 + 3) + 3 + 3 = 12

3. (a) What is α-complementation? How it has been used in gene cloning? [(CO2)(Explain/IOCQ)]

(b) Compare the use of following in gene cloning: cosmid, phagemid, YAC, MAC. [(CO3)(Understand/LOCQ)]

6 + 6 = 12

Group - C

4. (a) Write the names of two different popular biomolecules which we use as label in preparation of non-radioactive probe**.** Explain the steps of detection of a target DNA with a ss-DNA probe (assume which is labelled with anyone of the biomolecules you mentioned) using a chromogenic substrate, with diagram. [(CO2)(analyse)/ IOCQ)]

(b) Explain the steps of the technique to confirm the expression of a cloned gene in an expression vector at translational level with a diagram. [(CO2)(analyse)/ IOCQ)]

(c) Three restriction endonucleases (*SalI, EcoRI* and *HindIII*) are used to cut a plasmid DNA separately singly and in different combinations. From the agarose gel electrophoresis result, sizes of fragments (in kb) are listed in order of size. Determine the correct order of restriction sites, and draw the final restriction map of the DNA, with the intervals between sites labelled. ***SalI*) 20; *SalI*x*EcoRI*)** 7,13; ***SalI* x *HindIII*)** 4,5, 11; and ***SalI* x *EcoRI*x*HindIII*)** 3, 4, 5, 8. [(CO2)(analyse/ HOCQ)]

(1 + 4) + 4 + 3 = 12

5. (a) Explain the steps of standard PCR for amplification of DNA with diagram. [(CO3)(Remember/LOCQ)]

(b) Explain the principle, steps and purpose of Hot start PCR, with diagram. [(CO3)(Understand/ IOCQ)]

(c) Explain the principle, steps and purpose of Nested PCR, with diagram. [(CO3)(analyse/ HOCQ)]

4 + 4 + 4 = 12

Group – D

6. (a) Describe the technique only by labelled diagram, how you will purify eukaryotic mRNA. [(CO4)(Remember/IOCQ)]

(b) How pET vector system is used in preparation of cDNA library? What is its advantage? [(CO4)(Understand/HOCQ)]

6 + 6 = 12

7. (a) What is gene library? Schematically explain the construction of a genomic DNA library. [(CO4)(Remember/LOCQ)]

(b) Why gDNA library of a prokaryotic organism is often constructed by partial digestion of two different enzymes? [(CO4)(Understand/HOCQ)]

(2 + 6) + 4 = 12

Group – E

8. (a) Write the names of two genetic diseases with the names of their corresponding genes. Now explain the steps of treatment by gene therapy for anyone of the single gene defective genetic disease you have mentioned. [(CO5)(Explain/IOCQ)]
   (b) Write the names to human 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)]
   (c) In a biotech company, someone wants to clone a gene-X (whose protein product can be used a biopharmaceutical) from 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 + 4) + (1 + 3) + 3 = 12

9. (a) Explain the steps for production of the recombinant peptide vaccine against SARS-Cov2, with labelled diagram. [(CO5)(Explain/LOCQ)]
   (b) What are the different applications of genetic engineering? [(CO5)(Remember/LOCQ)]
   (c) Explain the principle and steps for the development of an insect resistant plant with a labelled diagram. [(CO5)(Explain/HOCQ)]

(1 + 4) + (2 + 5) = 12

Cognition Level	LOCQ	IOCQ	HOCQ
Percentage distribution	29.16	42.70	29.16

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 applications of recombinant DNA technology in different filed of biotechnology like gene therapy, human genome project, production of recombinant vaccine, explain the creation of transgenic animals and plants, construct recombinant biopharmaceutical, analyze and use of molecular biomarkers in disease diagnostics, forensic science with analysis of gene expression.
CO6. Analyze and solve problems related to rDNA technology.

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