RECOMBINANT DNA TECHNOLOGY
(BTC3103)

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) While preparing a cDNA library, the double stranded cDNAs are treated with DNA methylase to
(a) Methylate all restriction sites
(b) Methylate the linkers/adapters
(c) Methylate the internal restriction sites
(d) Methylate the vector

(ii) ATP, SAM and Mg2+ all are required by
(a) Type I restriction enzymes
(b) Type II restriction enzymes
(c) Both Type I and Type III restriction enzymes
(d) Both Type I and Type II restriction enzymes

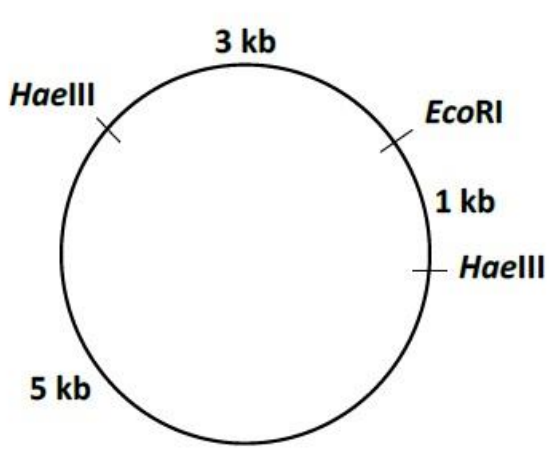
(iii) Match the techniques mentioned in group-I with their applications given in group-II.

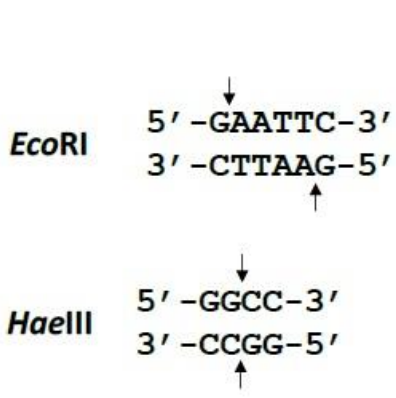
Group-I	Group-II
(P) PCR	(1) Identification of transcription factor binding sites in chromatin.
(Q)DNA microarray	(2) Identification of HIV infected patients.
(R) ELISA	(3) Identification of mouse homologue of a yeast gene.
	(4) Analysis of differential gene expression in cancer and normal cells.

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;

(iv) 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

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

(vi) The enzyme used for Blue-White screening:
(a) β-galactosidase
(b) IPTG
(c) X-gal
(d) All of these

(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) amp^r colonies; white colonies
(b) white colonies; amp^r colonies
(c) amp^r colonies, blue colonies
(d) blue colonies, amp^r colonies

(viii) Bacteria have been genetically modified for each of the following purposes except
(a) to produce human hormone insulin,
(b) to produce antibiotics that can be used to treat bacterial infection;
(c) to produce bacteria that can clean up toxic wastes such as oil spills;
(d) to produce enzymes will be used for industrial purposes these.

(ix) 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

(x) 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

Fill in the blanks with the correct word

(xi) The *E. coli* strain that must be used for pET expression vector system is _____.

(xii) *E.coli* DNA ligase needs _____ as cofactor.

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

(xiv) In the kinetics of end point of PCR deviates from linearity after _____cycle.

(xv) Human genetic disease that was successfully treated 1st time with gene therapy_____.

Group - B

2. (a) Compare the methods of screening bacterial clone with the following vector system: pBR322 vs pUC18
[(CO2)(Analyse/LOCQ)]

(b) Why host choice is the utmost importance in DNA cloning using pUC18 series of vectors?
[(CO2)(Understand/HOCQ)]

(c) Give one example of restriction enzyme with each of the following properties: (i) Tetracutter, (ii) 5' overhang producer, (iii) 3' overhang producer, (iv) blunt-end producer.
[(CO2)(Remember/LOCQ)]

6 + 2 +4 = 12

3. (a) Compare the following screening methods for selection of positive clones: Replica plating method vs Blue-White selection.
[(CO2)(Understand/IOCQ)]

(b) You have to amplify a DNA fragment by PCR whose primer contained SmaI sites only. But during cloning of the PCR product, you find out that your lab is running out of that enzyme. How can you clone the PCR product?
[(CO6)(Apply/HOCQ)]

(c) Name some commercially available reverse transcriptase. Discuss their application in recombinant DNA technology.
[(CO2)(Remember/LOCQ)]

4 + 4 + (1 + 3) = 12

Group - C

4. (a) Write the reaction mechanism for the polymerization of acrylamide to form polyacrylamide for SDS-PAGE.
[(CO3)(Remember/IOCQ)]

(b) Explain the principle of separation of nucleic acids by agarose gel electrophoresis (AGE), with a labelled diagram? Why separation of intact genomic DNA not possible by standard AGE? Explain the modified principle of electrophoresis to separate intact genomic DNA.
[(CO2)(Explain/IOCQ)]

(c) The *EcoRI* recognizes the sequence is 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 fragment after digestion. (Presume that 50% GC content in the genomic DNA).
[(CO6)(Apply/HOCQ)]

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

5. (a) Write the name of the best method of 1st generation DNA sequencing. Explain the principle and steps of method of DNA sequencing that you have mentioned, with a labelled diagram? *[(CO2)(Remember-Explain /HOCQ)]*

(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)(Explain/LOCQ)]*

(c) Three restriction endonucleases (RE-X, RE-Y and RE-Z) are used to cut a piece of linear DNA, singly and in pairwise combination. Sizes of fragments (in kb) are listed in order of size, *not* in linear order. Determine the correct order of restriction sites, and draw final restriction map, with the intervals between sites labelled. **X)** 11, 6, 5; **Y)** 14, 8 Z) 16, 6; and **X x Y)** 8, 6, 5, 3; **X x Z)** 11, 5, 5, 1; **Y x Z)** 8, 8, 6. *[(CO6)(Apply/IOCQ)]*

4 + 4 + 4 = 12

Group - D

6. (a) Discuss the steps of purification of plant gDNA with a flow diagram. *[(CO4)(Remember /LOCQ)]*
   (b) What are the role of CTAB, NaCl, βME and PVP in lysis buffer for plant gDNA isolation? *[(CO4)(Understand /IOCQ)]*
   (c) Which type of fusion protein is purified by Glutathione column? Explain the principle of that purification. *[(CO6)(Apply/IOCQ)]*

3 + 4 + (1 + 4) = 12

7. (a) What is gene library? Differentiate between gDNA and cDNA library. *[(CO4)(Analyse/LOCQ)]*
   (b) Describe with a flow chart the main steps of construction of both gDNA and cDNA library. *[(CO4)(Remember/IOCQ)]*
   (c) Why the cDNA library of a prokaryotic organism is often constructed by partial digestion of two different enzymes? *[(CO2)(Apply/HOCQ)]*

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

Group - E

8. (a) 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)(Explain/IOCQ)]*
   (b) Write the names of two single gene defective genetic disease with the name of defective gene. Now explain the steps of treatment of anyone of the single gene defective genetic disease you have mentioned by gene therapy, with labelled diagram? *[(CO5)(Explain/IOCQ)]*
   (c) In a biotech company, someone wanted to clone a gene-X (whose protein product can be used a biopharmaceutical) from eukaryote. A cDNA library is made from mRNA isolated from the corresponding eukaryotic cell. The number of mRNA corresponding to gene-X has a concentration of 10 molecules per cell. Each cell contains total 30,000 mRNA molecules. How many clones need to be screened from the cDNA library to find at least one recombinant clone containing a cDNA copy of gene-X with 95% probability? *[(CO6)(Apply/HOCQ)]*

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

9. (a) Write names of three biopharmaceutical? Explain principle and steps of cloning of the gene corresponding to anyone of the three biopharmaceuticals that you have mentioned, with diagram. *[(CO5)(Rember-explain/IOCQ)]*
   (b) Explain the SARSCov2 detection principle and the steps based on the virus specific RNA, with labelled diagram. *[(CO5)(Explain/IOCQ)]*
   (c) In human genome project, during making of genomic library, in a transformation experiment you used 10µl (amount 100 ng) ligated DNA aseptically added to 50 µl competent *E.coli* DH5α cell in a 1.5 ml tube. Then heat shock was given at 42^0C for 2 minutes. After that 0.95 ml LB was added to the tube. The tube was incubated in a shaker incubator for 30 minutes at 37^0C. Then 50µl solution from the tube was spreaded in a LA plate with ampicillin and incubated at 37^0C for overnight. Next day, number of transformant colonies were found which was equal to the last 2 digits of your autonomy roll number in your admit card. Now calculate the transformation efficiencies of this experiment. *[(CO6)(Apply/HOCQ)]*

(1 + 4) + 4 + 3 = 12

Cognition Level	LOCQ	IOCQ	HOCQ
Percentage distribution	27.8	50	22.2