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) A segment of DNA that reads the same from forward and reverse direction is called

(a) Palindrome (b) complementary DNA
(c) plasmid DNA (d) linker DNA.

(ii) The benefit of using qPCR is given below. Which statements are correct?

(p) It shows increased fluorescence after every cycle.
(q) It shows aa continuous increase in the fluorescence.
(r) It can be used to determine the number of transcripts in a given sample.
(s) It overcomes the inherent bias of the end point PCR.

(a) (p) and (s) (b) (q), (r) and (s)
(c) (p), (q) and (s) (d) (q) and (r).

(iii) The enzyme used for 3' end labeling of DNA is

(a) Klenow fragment (b) DNA pol I
(c) Terminal transferase (d) Polynucleotide kinase.

(iv) 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.

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

(vi) Match the techniques mentioned in Column A with their applications given in Column B.

Column A	Column 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

(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.

(vii) The type II restriction-modification system has:

(a) One large multifunctional enzyme with both endonuclease and methylase activity.
(b) Two separate enzymes for restriction digestion and methylation activity.
(c) Two separate sites of recognition and digestion.
(d) None of the above.

(viii) Which of following techniques will help to confirm the molecular weight of the purified protein?

(a) Ion exchange chromatography
(b) Affinity chromatography
(c) Hydrophobic interaction chromatography
(d) Gel filtration chromatography.

(ix) The enzyme responsible for blue-white screening is

(a) β galactosidase (b) IPTG
(c) X-gal (d) none of these.

(x) The enzyme used for 5' end labelling of DNA is

(a) Klenow fragment (b) DNA pol-I,
(c) Polynucleotide kinase (d) Terminal deoxynucleotide transferase.

Group – B

2. (a) Compare the suitability of T4 and T7 DNA polymerases for different applications.

   (b) For gene cloning experiments, why is the cleaved vector often treated with alkaline phosphatase prior to ligation step?

6 + 6 = 12

3. (a) Define plasmid. Why it is not considered as genome?

   (b) Enlist the characteristics required for plasmids to be used as ideal cloning vector.

   (c) Discuss the advantages and disadvantages of lambda phage as a vector.

3 + 6 + 3 = 12

Group – C

4. (a) Describe the chromogenic detection of the biotin labelled DNA probe with diagram.

   (b) What is the difference between normal PCR and QPCR? Explain the mechanism of the real-time PCR with TaqMan^{R} probe with diagram.

   (c) An aliquot of template DNA containing 2×10^{4} copies of target gene is placed into PCR reaction. The reaction has a mean efficiency of 95%. How many cycles are required to produce 2×10^{10}.

3 + (2 + 4) + 3 = 12

5. (a) What is site directed mutagenesis (SDM)? Describe method of SDM using PCR with a labelled diagram.

   (b) Write the names of different types of ELISA? Describe any one type of ELSA that you mentioned with diagram.

   (c) You have a 11 kb DNA template, in which only 1 kb inner sequence of the DNA in known and the flanking 5 kb sequences of the both side of known sequence is unknown. Now describe the steps and strategy to amplify the unknow sequence of this DNA by PCR. What is the name of this PCR technique?

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

Group – D

6. (a) What is genomic DNA library?

   (b) Describe preparation of genomic DNA library by schematic diagram.

   (c) What are the purposes for which genomic DNA library is constructed?

3 + 6 + 3 = 12

7. (a) Discuss the cloning strategy in: pBR 322 and pUC18

(b) What is the difference in screening?

(3 + 3) + 6 = 12

Group – E

8. (a) What is DNA vaccine? Describe principle and the method of production of DNA vaccine against Corona virus, with labelled diagram.

(b) Describe the principle and steps of development of insect resistant transgenic plant.

(c) A researcher desires to construct a genomic library of a microorganism for genome sequencing. Its genome size is 5×10^4 kb. The average size of its library fragment is 5 kb. The genomic library was created in vectors that were transformed into bacterial cells. If there is a 95% probability of the transformation, how many recombinant bacterial colonies will have to be screened to find this particular gene?

(1 + 4) + 5 + 2 = 12

9. (a) What are the different types of gene therapy based on the types of cell it can be done? Describe the principle and steps of in-vivo gene therapy that was done in case of curing of Cystic fibrosis, with labelled diagram.

(b) Write names of different molecular biomarker used in DNA finger printing. Describe the PCR-RFLP with example.

(c) What is the name of high throughput automated version of Northern blotting hybridization? Describe the principle and steps of those techniques with labelled diagram.

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

Department & Section	Submission Link
BT	https://classroom.google.com/w/MTE4ODk5NzQ1OTU4/tc/Mjc0ODM2ODc5NjQ2