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) The enzyme used for 3′ end labeling of DNA is
(a) Klenow fragment (b) DNA pol I
(c) Terminal transferase (d) Polynucleotide kinase.

(ii) Genomic DNA isolated from a bacterium was digested with a restriction enzyme that recognizes a 6-base pair (bp) sequence. Assuming random distribution of bases, the average length (in bp) of the fragments generated is
(a) 4906 (b) 4096 (c) 4609 (d) 4960.

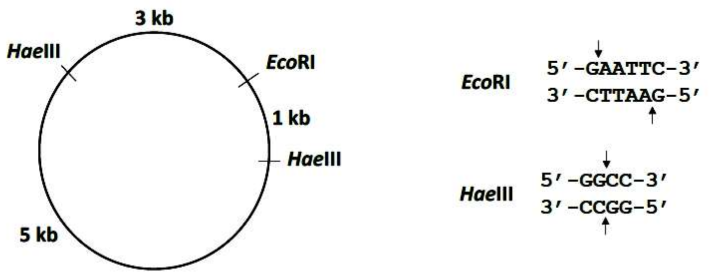
(iii) A gene was cloned into a unique HindIII restriction site present in the ampicillin resistance gene of a vector that contains both ampicillin and kanamycin resistance genes. To select for only recombinant clones, the transformation mixture should be plated on which of the following plates?
(a) Ampicillin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

(iv) If you start with 6 copies of a DNA molecule, and perform six cycles of standard PCR, how many double stranded copies of the starting material will you get?
(a) 834 (b) 483 (c) 384 (d) 84.

(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

B.TECH/BT/5TH SEM/BIOT 3103/2018

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 most preferred enzyme for Sanger's dideoxy DNA sequencing is
(a) Klenow fragment (b) T4 DNA polymerase
(c) T7 DNA polymerase (d) Taq polymerase.

(vii) Which one of the following is a goal of the Human Genome Project?
(a) Analyze the genomes of model organisms.
(b) Obtain the DNA sequence of the entire human genome.
(c) Develop programs that focus on understanding and addressing the ethical, legal and social implications of the results obtained from the Human Genome Project.
(d) All of the above.

(viii) In a typical gene cloning experiment, by mistake a researcher introduced the DNA of interest within the ampicillin resistance gene instead of lacZ gene. The competent cells were allowed to take up the plasmid and then plated on the media containing ampicillin, X-gal and IPTG and subjected to blue-white screening. Considering all plasmids were recombinant which one of the following statements correctly describes the outcome of the experiment?
(a) The bacteria which took up the plasmids would grow and give blue colonies.
(b) The bacteria which took up the plasmids would not grow.
(c) The bacteria which took up the plasmids would form white colonies.
(d) All of the bacteria would grow and give white colonies.

(ix) The following table provides information about four proteins. Which one of the following options correctly identifies the order of elution in size exclusion chromatography and the increasing order of mobility in SDS polyacrylamide gel?

Protein	Native mol. wt. (Da)	pI	Type
P	32000	6.4	monomer
Q	40000	8.5	homodimer
R	25000	4.9	monomer
S	45000	8.5	homotrimer

(a) Chromatrography: SQPR; Electrophoresis: RPQS
(b) Chromatrography: RPQS; Electrophoresis: SQPR
(c) Chromatrography: PRQS; Electrophoresis: PRQS
(d) Chromatrography: SQPR; Electrophoresis: PRQS

(x) Diagnosis of influenza virus infections can be done using some of the following techniques:
P) Western blot and Southern blot Q) Northern blot and western blot
R) ELISA and RT-PCR S) PCR and electron microscopy
Choose the combination of techniques that correctly lists the detection methods.
(a) P and R only (b) R and S only
(c) Q and R only (d) P and S only.

Group – B

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

(b) What are the most commonly used alkaline phosphatases for recombinant DNA experiments? For gene cloning experiments, why is the cleaved vector often treated with alkaline phosphatase prior to ligation step?

6 + (2 + 4) = 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 with labelled diagram and example.

(c) Discuss the advantages and disadvantages of lambda phage as a vector over pBR 322.

(1 + 2) + 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 3×10^4 copies of target gene is placed into PCR reaction. The reaction has a mean efficiency of 85%. How many cycles are required to produce 2×10^{10}.

3 + (1 + 4) + 4 = 12

5. (a) Write the name of is the best method of 1st generation DNA sequencing. Describe the principle and steps of that method of DNA sequencing with a labeled diagram.

(b) Describe the principle and steps of N-terminal sequencing of protein by Edmann degradation method with a labeled diagram.

(c) What is site directed mutagenesis (SDM)? Describe a method of SDM by PCR with a labeled diagram.

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

Group – D

6. (a) Discuss the purification process of plant gDNA. What is the role of CTAB here?

(b) Discuss the process of construction of gDNA library by a flow chart.

6 + 6 = 12

7. (a) Describe the steps for the cloning of a gene X from prokaryotic organism into PUC18 with single restriction enzymes, with diagram.

(b) Why the efficiency of getting positive clone by above method will be low? What are the different ways in which you can improve the cloning efficiency of this method? Describe with diagram.

(c) Describe the selection of positive clone by pUC18 and the mechanism of selection.

5 + (2 + 3) + 2 = 12

Group – E

8. (a) What was the goal of Human Genome Project (HGP)? What sequencing principle was used in HGP? Describe that principle with a labled diagram.

(b) Describe the steps and principle of 2nd generation DNA sequencing developed by 454 life sciences with a labeled diagram.

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

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

9. (a) What is DNA vaccine? Describe the method of production of DNA vaccine against a antigen, with labeled diagram.

(b) What are the different applications of genetic engineering? Describe the method of development of an insect resistant plant.

(c) Why over expression of eukaryotic gene is problematic in prokaryote? How these problem can be solved?

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