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 mixture contains three similarly sized peptides P, Q and R. The peptide P is positively charged, Q is weakly negative and R is strongly negative. If this mixture is passed through an ion-exchange chromatography column containing an anionic resin, their order of elution will be
(a) P, Q, R; (b) R, Q, P;
(c) Q, R, P; (d) P, Q and R elute together.

(ii) A plasmid DNA when digested with EcoRI gave a single band of 16 Kb. When the same plasmid was digested with BamHI it gave two bands of 6Kb and 4 Kb. The plasmid has
(a) Single site of EcoRI and 2 sites of BamHI
(b) Single site of EcoRI and 3 sites of BamHI
(c) Single site of EcoRI and 2 sites of BamHI
(d) 2 sites of EcoRI and 2 sites of BamHI

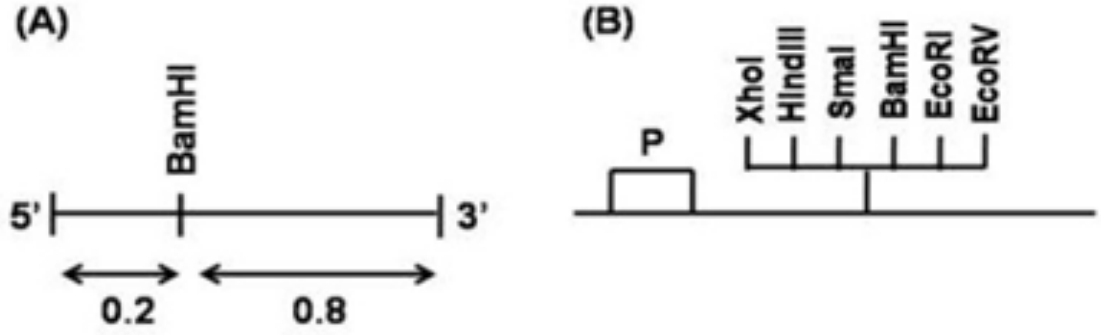
(iii) Which of the following components terminates the chain in a Sanger's sequencing reaction?
(a) deoxynucleotides (b) Taq polymerase
(c) oligonucleotide primer (d) dideoxynucleotide.

(iv) Given below are two sets of terms related to various methods used in genetic engineering

Group-I		Group-II	
P	Steptavidin	(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).

(v) In an experiment, a 1kb fragment with a single BamHI site (as shown in figure 'A') is to be cloned in the SmaI (CCCGGG) site of a cloning vector of 3 kb length (figure 'B'. None of the other enzymes of the multiple cloning site are present in the fragment to be cloned.



Based on the information given above, a series of digestion were set up for the potential clones and their fragment profile are given below

(P) BamHI : 200 bp + 3.8 kb
(Q) BamHI : 800 bp + 3.2 kb
(R) HindIII + EcoRI : ~1 kb + ~ 3 kb
(S) XhoI + BamHI : ~200 kb + ~ 800 kb + ~ 3 kb

Which one of the above digestion profiles confirms successful cloning of the fragment in the vector in an orientation wherein the 5' end of the cloned fragment is towards 'P'?

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

(vi) 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) 40,096 bp (b) 4.096 kb
(c) 4096 kb (c) 4906 bp

(vii) The tetanus vaccine given to human in the case of a deep cut is a

(a) DNA vaccine (b) recombinant vaccine,
(c) subunit vaccine (d) toxoid vaccine.

(viii) A researcher desires to clone a gene of a microorganism. Its genome size is 1.5x 10^4kb. 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?

(a) 7000 (b) 8000
(c) 9000 (d) 10000.

(ix) Which of the following techniques is not involved in the identification of DNA at crime scenes against possible suspects?

(a) ELISA (b) western blot
(c) Northern blotting (d) DNA fingerprinting.

(x) A contig is
(a) A map of genetic markers that separated by less than 1cM.
(b) a map showing the order of cloned bits of DNA.
(c) unique DNA sequences that serve as molecular markers.
(d) sets of two or more partially overlapping cloned DNA fragments.

Group – B

2. (a) What are restriction endonucleases? Explain why type II restriction endonucleases are preferred to other classes for recombinant DNA technology.

(b) What is Klenow fragment? Describe the major applications of Klenow fragment.

(2 + 4) + (2 + 4) = 12

3. (a) What are the basic features of a standard plasmid?

(b) What are: (i) cloning vector and (ii) expression vector.

(c) How reverse transcriptase is different from Klenow fragment?

4 + 4 + 4 = 12

Group – C

4. (a) To get the positive clone for DNA sequencing of a gene of interest a researcher did the following procedure. The 50 mL of competent *E.coli* cells (10^9 CFU/mL) were transformed using 0.5ng of a plasmid DNA to which 950 mL of SOC medium was added. Only 50 µL of this was plated on a selective agar plate. After a 12hr incubation at 37°C, 90 colonies were observed. Calculate the efficiency of this transformation in *CFU/µg* of DNA.

(b) The positive clone was confirmed by western blotting hybridization technique. Describe that technique using anon-radioactive detection probe with label diagram.

(c) 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 by EcoRI and XhoI gives you fragments of 2, 3, and 5 kb. From these results, you deduce restriction map of the plasmid.

(d) Starting with 500 template DNA molecules after 30 cycles of PCR, how many molecules of DNA will be produced?

2 + 5 + 3 + 2 =12

5. (a) Describe a method of preparation of random, 5'-end and 3'-end labelled radioactive probe only by label diagram.

(b) PCR typically used to amplify DNA that lies between two known sequences, then how you will amplify the end sequences of DNA whose internal sequences is known to you?

(c) Then cloned DNA was sequenced with a method sequencing without the electrophoresis but using bioluminescence. Describe that technique of DNA sequencing by label diagram

(d) The restriction endonuclease EcoRI recognizes the sequence GAATTC. If a 40.96 kb genomic DNA with random sequence digested with EcoRI, theoretically how many fragments will be produced? (Presume that 50% GC content in the genomic DNA).

3 + 3 + 4 + 2 = 12

Group – D

6. (a) How mRNA is isolated and purified from total cellular RNA?

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

6 + 6 = 12

7. (a) Discuss the cloning strategy in: pBR322 and pUC18

(b) What is the difference in screening?

(3 + 3) + 6 = 12

Group – E

8. (a) Describe a flow chart for cloning of human growth hormone(hGH) gene to produce recombinant hGH.

(b) Describe two detection methods for identify pathogenic virus.

(c) Describe the steps to cure SCID by gene therapy.

4 + 4 + 4 = 12

9. (a) Write an account on human genome project. How it is helpful to mankind?

(b) What are the basic features of siRNA and how are they used in gene therapy?

(c) A researcher made a genomic library of microorganism for sequencing of the gene in a genome project. Its genome size is 4.2 x 10^6 kb. The average insert size of its genomic library fragment is 4 kb. The genomic library was created in vectors that were transformed into bacterial cells. If there is a 92% probability of the transformation, how many recombinant bacterial colonies will have to be screened to find this particular gene?

5 + 5 + 2 =12

Department & Section	Submission Link
BT	https://classroom.google.com/w/MTE4ODk5NzQ1OTU4/tc/Mjc0ODM2ODc5Njk1