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) If you start with 10 copies of dsDNA molecules, and perform six cycles of standard PCR, then how many double stranded copies of the starting molecule will you get at the end?
(a) 320 (b) 640, (c) 460, (d) 64

(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) 4609 (c) 4096 (d) 4960

(iii) Why are gene libraries constructed?
(a) To find new gene,
(b) To sequence whole genome
(c) To create a "bank"of the genes in an organism
(d) All of the above.

(iv) A polymerase reaction is carried out for 10 cycles in a volume of 1 ml with 5 molecules of template DNA. Assuming that the efficiency of the reaction is 100 %, the number of molecules of DNA present in 10 μl at the end of the reaction is ____.
(a) 12.5, (b) 51.2, (c) 21.5, (d) 25.1

(v) What are the components of CRISPR/Cas9
(P) Guide RNA, (Q) Spacer,
(R) PAM sequence, (S)Target sequence
(a) (P) and (Q), (b) (P), (Q) and (R),
(c) (P) and (S), (d) (P), (Q), (R) and (S)

(vi) How are restriction enzyme (RE) and T4DNA ligase used in genetic engineering?
(a) Restriction enzyme cut the DNA at specific site, producing ends that can be ligated back together with ligase.
(b) Only restriction enzyme that produces blunt ends after cutting DNA can be ligated with ligase.
(c) Only restriction enzyme that produces sticky ends on the DNA can be ligated with ligase.
(d) Restriction enzyme randomly cuts DNA and the cut fragment can be ligated back together.

(vii) Which of the following DNA detection techniques is not based on principle of fluorescence spectroscopy?
(a) qPCR with TaqMan probe (b) FISH
(c) qPCR with hybridization probe (d) End point PCR

(viii) Why are subtractive cDNA libraries constructed?
(a) To study gene expression
(b) To sequence whole genome
(c) To create a "bank"of the genes in an organism
(d) To find out gene expressed in different conditions

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

(x) Which of the following enzyme does not require a template for polymerization:
(a) Reverse transcriptase (b) Taq DNA polymerase
(c) Terminal transferase (d) DNA pol I

Group- B

2. (a) What are the different R-M systems? Which of them is preferred in rDNA technology and why? [(CO1) (Analyze/IOCQ)]

(b) Write thereactionscatalysed by the following enzymes labelled diagram:
(i) Klenow
(ii) BAP. [(CO1) (Remember/LOCQ)]

(c) What is the relationship between:
i) Bam HI and Bgl II
ii) Sac I and Sst I. [(CO2) (Correlate/IOCQ)]

4 + 4 + 4 = 12

3. (a) Compare the basic features of the following with suitable example:
(i) cloning vector and (ii) expression vector. [(CO4)(Compare/HOCQ)

(b) Discuss the advantages and disadvantages of lambda phage as a vector.
[(CO4)(Analyze/IOCQ)] **(3 + 3) + 6 = 12**

Group - C

4. (a) Explain the principle of DNA sequencingby pyrosequencing methods, with labelled diagrams. Why this method called pyrosequencing? [(CO2) (Explain/LOCQ)]

   (b) Write three differences between Sanger dideoxy methods and pyrosequencing methods. [(CO2) (Differentiate/IOCQ)]

   (c) Explain the mechanism of the enzymatic reaction, based on which pyrosequencing method is developed? [(CO2) (Understand/LOCQ)]

   (d) You a solution with 25 template DNA molecules. How many cycles of PCR you have to run to get 10^{25} copies of DNA? [(CO3) (Evaluate/HOCQ)]

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

5. (a) What is the principle of separation of nucleic acids by agarose gel electrophoresis (AGE)? Why separation of intact genomic DNA not possible by standard AGE? Describe the modified principle of electrophoresis to separate intact genomic DNA. [(CO2)(Explain/LOCQ)]

   (b) Write the reaction mechanism for the polymerization of acrylamide to form polyacrylamide. [(CO2) (Understand/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 the map, with the intervals between sites labelled. X) 11, 6, 5; Y) 14, 8 Z) 16,6; and XxY) 8, 6, 5, 3; XxZ) 11, 5, 5, 1; Y x Z) 8, 8, 6. [(CO6) (analyse/IOCQ)]

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

Group – D

6. (a) What are the problems of cloning DNA into a vector, when DNA and vector both are digested with single restriction enzyme? How these problems can be solved? Describe with diagram. [(CO4) (Analyze/IOCQ)

   (b) Describe any one selection technique for positive clones containing of insert DNA. [(CO5) (Understand/LOCQ)]

(3 + 4) + 5= 12

7. (a) Discuss the DNA cloning strategy with the following vectors (i) pBR322 and (ii) pUC18. [(CO2)(Compare/IOCQ)

   (b) What is the difference in screening of recombinant for cloning process with the above two vectors? [(CO2)(Differentiate/IOCQ)]

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

Group - E

8. (a) What is biopharmaceutical? Describe cloning principle and steps of hGH gene for production of human recombinant growth hormone, with diagram. [(CO5) (Understand/IOCQ)]

   (b) Explain the detection principle and steps forSARSCov2 with labelled diagram, based on specific protein of the virus. [(CO5) (Analyse/IOCQ)]

   (c) Explain theprinciple and steps of creation of GMO "superbug" with labelled diagram. [(CO5) (Understand/LOCQ)]

   (d) In human genome project, during making of genomic library,in a transformation experiment, 0.1 ml of *E.coli* competent cells was added with 15 ng of ligated DNA (containing a recombinant plasmid with amp^R gene) in a tube. Then, it was kept in ice for 5 min. Then, heat shock was given at 42°C for 2 min. Then, 0.9 ml of LB media was added to it and then the tube was incubated for 30 min at 37°C temperature, in a shaker incubator before plating. Then, 0.1 ml transformed cells were spread on LB-agar plate containing ampicillin (100 μg/ml) and incubated at 37°C incubator for overnight. Next day, you observed colonies were present in the plate and when you counted the colonies, you found that number colonies present in the plate was equal to your 250. Now you evaluate the transformation efficiency of the experiment? [(CO6) (Evaluate/HOCQ)]

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

9. (a) Explain the different strategies of human genome sequencing in HGP? [(CO5) (Illustrate/IOCQ)]

   (b) What are the basic features of siRNA and how are they used in gene therapy? [(CO5) (Remember/LOCQ)]

   (c) A researcher made a genomic library of microorganism for sequencing of the gene in a genome project. Its genome size is 4.2×10^6 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 90% probability of the transformation, how many recombinant bacterial colonies will have to be screened to find this particular gene? [(CO6) (Evaluate/HOCQ)]

4 + (2 + 3) + 3 = 12

Cognition Level	LOCQ	IOCQ	HOCQ
Percentage distribution	28%	52%	20%

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

Department & Section	Submission Link
BT	https://classroom.google.com/c/NDA0NzA2MTczODIz/a/NDY0MjMyOTU3NDc5/details