M.TECH/BT/1ST SEM/BIOT 5101/2021

ADVANCED GENETIC ENGINEERING
(BIOT 5101)

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 mRNA coding for a secretory protein, when translated using free ribosome under *in vitro* conditions, resulted in a 40 kDa protein. The same mRNA when translated using therough endoplasmic reticulum resulted in a 36 kDa protein. The difference in the molecular weight of the two polypeptides is due to the loss of a
(a) 2 kDa peptide from N-terminus and a 2 kDa peptide from the C-terminus
(b) 1 kDa peptide from N-terminus and a 3 kDa peptide from the C-terminus
(c) 4 kDa peptide from the N-terminus
(d) 4 kDa peptide from the C-terminus

(ii) 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) no colonies; white colonies.

(iii) Which of the following techniques is not involved in the identification of DNA at crime scenes against possible suspects?
(a) PCR (b) Western blot
(c) DNA Sequencing (d) DNA fingerprinting

(iv) (i) 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.

(v) Restriction endonuclease generated DNA fragments separated by gel electrophoresis and blot transferred onto a membrane filter are probed with a radioactive DNA fragment. This procedure is called:
(a) Gene cloning (b) Recombinant DNA
(c) The polymerase chain reaction (d) The Southern technique

(vi) Pure plasmid DNA was isolated from a bacterium. Restriction enzyme digestion of this plasmid with either *Hind III* or *BamH* I resulted in two DNA fragments. A double digestion of the same plasmid with both these enzymes resulted in three DNA fragments. From this we can conclude that the isolated plasmid DNA is
(a) Single stranded and circular (b) Single stranded and linear,
(c) Double stranded and circular (d) Double stranded and linear

(vii) In a qRT-PCR experiment for quantitation of unknown RNA from a COVID19 patient sample, the $\mathbf{C_T}$ value was 12. What was amount of RNA copies present in the unknow sample?
(a) 4.096 copies (b) 2048 copies (c) 8192 copies (d) 4096 copies

(viii) Biopharmaceuticals are classified into groups. **Match the Group-I and group-II**

Group-I	Group-II
(P) Protein therapeutics with enzymatic or regulatory activity	(1) Hepatitis B surface antigen
(Q) Protein therapeutics with special targeting activity	(2) Insulin aspart
(R)Protein vaccines	(3) Secretin
(S) Protein diagnostics	(4) Transtuzmab

Which one of the following options represents correct match of group-I and Group-II?
(a) P – 2; Q – 4; R – 1; S – 3 (b) P – 1; Q – 2; R – 3; S–4
(c) P – 3; Q – 4; R – 2; S – 1 (d) P – 4; Q – 1; R – 3; S - 2

(ix) Single stranded T-DNA from Ti plasmid in *Agrobacterium* is produced by the action of
(a) *chvA* and *chvB* (b) *vir* D1 and *vir* D2
(c) *vir* E1 and *vir* E2 (d) *vir* B1-B11

(x) A chimeric promoter is
(a) promoter regions taken from many different systems

(b) promoter region generally taken and fused from 2 different systems
(c) promoter region taken from CaMV
(d) none of these

Group–B

2. (a) Write three the differences between normal PCR and QPCR. [(CO1) (Understand/LOCQ)]
   (b) Analyze the real-time fluorescent PCR technique with molecular beacon probe. [(CO1) (Analyze/IOCQ)]
   (c) In the process of cDNA library screening if you have ended up getting partial clones, how would you complete these partial clones into full length by PCR? [(CO2) (Explain/IOCQ)]
   (d) An aliquot of template DNA containing 3×10^2 copies of target gene is placed into PCR reaction. The efficiency of thermal cycler is 90%. Evaluate, how many cycles are required to produce 2×10^{10} copies of DNA? [(CO6) (Solve/HOCQ)]

3 + 3 + 4 + 2 = 12

3. (a) Describe the detection of DIG-labelled probe by chromogenic detection method by label diagram. [(CO4) (Understand/LOCQ)]
   (b) Enumerate the steps of western blotting technique with a labelled diagram, and write its application and disadvantages. [(CO3) (Analyze/IOCQ)]
   (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) (Calculate/HOCQ)]

3 + (3 + 1.5 + 1.5) + 3 = 12

Group - C

4. (a) What are the parameters one must take into account when designing the primer for PCR amplification? [(CO1)(Remember/LOCQ)]
   (b) As PCR is typically used to amplify DNA that lies between two known sequences, then explain the steps of amplifying the end sequences of DNA whose internal sequences are known to you. [(CO1)(Understand/LOCQ)]
   (c) You are trying to create a restriction-map of a plasmid DNA. A *BamH*I digest gives you a 10 kb fragment, and *Hind*III digest gives you a 2 kb and an 8 kb fragment, and the double digest by *BamH*I and *Hind*III gives you fragments of 2, 3, and 5 kb. Deduce restriction map of the plasmid from these results. [(CO6)(Deduce/HOCQ)]

4 + 4 + 4 = 12

5. (a) With a labelled diagram, describe a DNA cloning method without the use of restriction and DNA ligase. [(CO2)(Understand/LOCQ)]

(b) Analyze the steps of making cDNA library of a eukaryotic cell with a labelled diagram. [(CO3)(Analyze/IOCQ)]

(c) The rarest mRNA in a cell of a particular type has a concentration of eight molecules per cell. Each cell contains 50,000 mRNA molecules. A cDNA library is made from mRNA isolated from this tissue. How many clones will need to be screened to have a 95% probability of finding at least one recombinant containing a cDNA copy of rarest mRNA? [(CO6)(Analyze/IOCQ)]

4 + 5 + 3 = 12

Group – D

6. (a) Where ES can be found? Why it is preferred for viral transduction? [(CO5)(Remember/LOCQ)]

(b) Compare and contrast the following methods for raising transgenic plants and animals:
(i) LASER mediated
(ii) Viral vector mediated. [(CO5)(Distinguish/IOCQ)]

6 + (3 + 3) = 12

7. (a) Discuss the steps for Biolistic gene delivery system with diagram. [(CO5)(Remember/LOCQ)]

(b) What is shuttle vector? Why it is called so? [(CO5)(Analyze/IOCQ)]

6 + (3 + 3) = 12

Group – E

8. (a) Explain the cloning of human TPA gene for the production of rh-tissue plasminogen activator, with labelled diagram. [(CO2)(Remember/LOCQ)]

(b) Describe principle and steps of the techniques of DNA finger printing to solve the parenting problem? [(CO5)(Explain/IOCQ)]

(c) In a gene expression analysis experiment using qRT-PCR, for tumour cell and control cell sample, the following results were obtained.

	Gene	
Sample	CT of p53 (target)	CT of GAPDH (reference)
Control cell (calibrator)	14	15.5
Tumour cell (test)	10	16.5

Calculate the fold change in expression of p53 gene of tumour cell with respect to the control cell by Livack method. [(CO6)(Analyze/IOCQ)]

(d) You have planned to make a genomic library using λEMBL3 vector, using Sau3A1 partial digest of the human genome (3 x 10^9 bp). If you wish a 99% chance of isolation of the desired gene from the λEMBL3 genomic library by screening of 688,00 independent clones, then evaluate what was the insert size of the library? [(CO6)(Evaluate/HOCQ)]

4 + 3 + 3 + 2 = 12

9. (a) Mention the names of different types of vaccine according to their nature. Assume that you got a job in a biotech company and you are asked to prepare a plan for preparation of peptide vaccine against SARS-Cov2. Explain the logic and the steps of your plan to prepare the peptide vaccine with a diagram. [(CO6)(Apply/HOCQ)]

(b) Write names of three genetic diseases. How you will do DNA based diagnosis of anyone of those genetic diseases? Give a logical explanation of the steps with diagram. [(CO6)(Explain/IOCQ)]

(c) Write the names of three biopharmaceuticals. How you will produce anyone of those biopharmaceuticals, explain the logic and steps with diagram. [(CO6)(Explain/IOCQ)]

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

Cognition Level	LOCQ	IOCQ	HOCQ
Percentage distribution	35%	49%	16%

Course Outcome (CO):

After the completion of the course students will be able to:

1. Describe the function and application of the common enzymes used in molecular biology and explain the different DNA sequencing methods and when they would be applied.
2. Explain which biological hosts is the best choice for producing a certain protein and why.
3. Give examples of how to increase or decrease the expression of a given gene using gene regulation mechanisms.
4. Describe methods for performing DNA mutagenesis and how to screen or select for successful mutants.
5. Apply to produce of transgenic plants and animals and explain the principles behind modern gene therapy.
6. Apply the knowledge of genetic engineering in problem solving and in practice

*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/NDEwNjQ0NTY2MjY0/a/NDc0ODIyNTE5NzU2/details