M.TECH/BT/1st SEM/BIOT 5101/2017
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) 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.

(ii) The enzyme used for 3' end labeling of DNA is
(a) Klenow fragment
(b) DNA pol-I,
(c) Polynucleotide kinase
(d) terminal deoxynucleotide transferase

(iii) In particle bombardment, theoretically
(a) any plasmid can be used (b) any explants can be taken
(c) any gene can be incorporated (d) all of the above

(iv) BAC can accommodate approximately
(a) 5 to 10 kb of insert
(b) 50 to 100 kb of insert
(c) less than 45 kb of insert
(d) more than 100 kb of insert

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

(vi) What type of chemical bond formation is catalyzed by DNA ligase?
(a) Glycosidic (b) Hydrogen (c) Phosphoester (d) Ester.

(vii) 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 gives you fragments of 2, 3, and 5 kb. From these results, you deduce that the size of the plasmid is _____ kb, the number of EcoRI sites is _____, the number of XhoI sites is _____, and that there is an EcoRI site within the _____ kb XhoI fragment.
(a) 10; 2; 1; 8 (b) 10; 1; 2; 8
(c) 20; 2; 2; 8 (d) 20; 1; 2; 8.

(viii) If you start with 2 copies of ds DNA molecules, and perform six cycles of standard PCR, how many double stranded copies of the starting material will you get?
(a) 32 (b) 128 (c) 64 (d) 16.

(ix) The 50 mL of competent *E.co/i* cells (10^9 CFU/mL) were transformed using 0.5 ng of a 5 kb plasmid DNA to which 950 mL of LB medium was added. Only 50 uL of this was plated on a selective agar plate. After 12 h incubation at 37°C, 90 colonies were observed. Calculate the efficiency of this transformation in *CFU/μg* of DNA.
(a) $3.6 \times l0^5$ (b) $3.6 \times l0^6$ (c) $1.8 \times l0^5$ (d) $1.8 \times l0^6$.

(x) A researcher desires to clone a gene (1 kb) of a microorganism. Its genome size is 1.5×10^4 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?
(a) 7000 (b) 8000 (c) 9000 (d) 10000.

Group – B

2. (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) Three restriction endonucleases are used to cut a piece of DNA, singly and in pairwise combination. Sizes of fragments are listed in order of size (kb), *not* in linear order. Determine the correct order of restriction sites, and draw the map, with the intervals between sites labelled.
   BamH1: 11, 6, 5; EcoRI:14,8; PstI:16,6; BamHI /EcoRI: 8, 6, 5, 3; BamH I/ PstI: 11, 5, 5, 1; EcoRI / PstI: 8, 8, 6.

3 + (1 + 4) + 4 = 12

3. (a) Describe automated DNA sequencing method with labelled diagram.

   (b) What are the different ways we can sequence RNA? Describe any one method of RNA sequencing with labelled diagram.

   (c) What are high capacity vectors? Describe a high capacity vectors with labelled diagram.

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

Group – C

4. (a) Describe the mechanism of control of over expression of cloned gene in pET vector system with diagram.

   (b) A pure protein X of a bacteria is available, describe the plan to clone the gene of protein X with a labelled diagram.

   (c) The rarest mRNA in a cell of a particular type has a concentration of five molecules per cell. Each cell contains 450,000 mRNA molecules. A cDNA library is made from mRNA isolated from this tissue. How many

clones will need to be screened to have a 99% probability of finding at least one recombinant containing a cDNA copy of rarest mRNA?

6 + 4 + 2 = 12

5. (a) What is primer? What is RT- PCR? How you will synthesize ds cDNA?

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

   (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} copies.

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

Group - D

6. (a) What are ES-cells? How are they produced?

   (b) Describe the transfection method to ES cells.

   (c) Describe the process of developing transgenic sheep Dolly in detail.

3 + 3 + 6 = 12

7. (a) What are Ti and Ri-plasmids? Where are they found?

   (b) Mention the functions of all *vir*-genes in the natural process of gene delivery of *Agrobacterium* to plant cells.

(2 + 2 + 2) + 6 = 12

Group - E

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

   (b) Describe two detection methods to identify pathogenic virus or bacteria.

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

4 + (2 + 2) + 4 = 12

9. Write Short notes about the following:
   (i) Human gene therapy
   (ii) Cloning of tPA gene
   (iii) DNA finger printing forensic science.

(4 × 3) = 12