#### M.TECH/BT/1ST SEM/BIOT 5101/2020

## 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 <u>any 5 (five)</u> from Group B to E, taking <u>at least one</u> from each group.

Candidates are required to give answer in their own words as far as practicable.

# Group – A (Multiple Choice Type Questions)

(i)	Choose the right combination of components required to set up a polymerase chain reaction from the following

- (a) Template DNA, two primers, dNTPs and DNA ligase
- (b) Template DNA, two primers, NTPs and DNA ligase

Choose the correct alternative for the following:

- (c) Template RNA, two primers, NTPs and DNA polymerase
- (d) Template DNA, two primers, dNTPs and DNA polymerase
- (ii) In an experiment of prokaryotic gene expression analysis by PCR, starting amount DNA was  $3 \times 10^5$  copies. If the efficiency of the PCR thermalcyclerwas 85% and the yield was  $2 \times 10^{10}$  copies of DNA, then how many numbers of cycles we ran the PCR? (a) 17 cycles (b) 2 cycles (c) 18 cycles (d) 17.8 cycles.
- (iii) A plasmid can be transformed into Agrobacterium by(a) CaCl<sub>2</sub>-phenol mediated gene delivery (b) Tri-Parental mating

(c) Electroporation

(d) All of these

 $10 \times 1 = 10$ 

- (iv) 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.
- (v) One physical method for gene transfer technique for animal is (a) Ca-phosphate mediated gene delivery (b) Sonication
  - (c) Electroporation (d) Liposome-mediated
- (vi) Which of the following can be used for transferring the DNA into the host cells?P. Transformation Q. Sonication R. Transfection S. Electroporation

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(a) Only P can be used

(b) Only Q & R can be used

(c) Only Q, R & S can be used (d) Only P, R & S can be used.

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(vii) Match between the terms related to human genome project (group-I) and with their definition (Group-II)

Group-I	Group-II
(P) Read	(1) a contiguous sequence formed by several overlapping reads
	with no gaps
(Q) Contig	(2) an ordered and oriented set of contigs, usually by mate pairs
(R) Mate pair	(3) a pair of reads from two ends of the same insert fragment
(S) Scaffold	(4) a 500-900 long word that comes out of sequencer

Which one of the following is the correct match between group-I and group-II

- (a) P 1; Q 2; R 3; S 4.
- (b) P 4; Q 1; R 3; S 2.

(c) P - 3; Q - 1; R - 2; S - 2.

(d) P - 1; Q - 4; R - 3; S - 2.

- (viii) pGREEN is a
  - (a) Cloning vector

(b) Micro Ti plasmid

(c) Promoter

- (d) Terminator
- (ix) A researcher desires to clone a gene of a microorganism. Its genome size is  $1.5 \times 10^4$ 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.

- (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) Describe the principles and steps of pyrosequencing methods of DNA sequencing with labelled diagrams only. Why this method called pyrosequencing?
  - (b) What are differences between Sanger dideoxy methods and pyrosequencing methods of DNA sequencing?
  - (c) Based on which important enzymatic reaction these above methods are developed? Write that reaction.
  - (d) Three restriction endonucleases (RE-D, RE-Eand RE-F) 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 final restriction map of the DNA, with the intervals between sites labelled.

**D)** 18, 5; **E)** 15, 7, 1; **F)** 20, 3; **D x E)** 10, 7, 5, 1; **D x F)** 15, 5, 3; and **E x F)** 15, 4, 3, 1.

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

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- 3. (a) Write the name of the techniques by which a cloned DNA can be sequenced without the electrophoresis but using bioluminescence. Describe that technique of DNA sequencing by label diagram.
  - (b) Write the enzymatic reaction based on which the above methods of DNA sequencing were developed.
  - (c) Describe the features of YAC and BAC vectors with labelled 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).

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

## Group - C

- 4. (a) What are the problems of cloning DNA into a vector, when DNA and vector both will be digested with single restriction enzyme? How these problems can be solved? Describe with diagram.
  - (b) Describe one selection technique for positive clones containing of insert DNA.
  - (c) Write the names of different techniques to clone DNA without the use restriction enzyme and DNA ligase. Describe any one technique by labelled diagram only.

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

- 5. (a) Describe the steps of making cDNA library.
  - (b) Describe a technique for screening of cDNA library.
  - (c) Describe the general features of expression vector with a labelled diagram.

$$4 + 4 + 4 = 12$$

### Group - D

- 6. (a) Describe the process of developing transgenic mice with a flow chart.
  - (b) Compare the advantages and disadvantages of the following methods for raising both transgenic plants and animals:
    - (i) PEG-mediated protoplast fusion, (ii) Microinjection

$$6 + (3 + 3) = 12$$

- 7. (a) What are *vir* genes? Discuss their role in transfer of T-DNA.
  - (b) Compare and contrast the advantages and disadvantages of Agro-mediated gene delivery with Particle bombardment.

$$(2+6)+4=12$$

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## Group - E

- 8. (a) Discuss the aims and objectives of Human Genome Project.
  - (b) Describe the two methods of genome sequencing used for HGP.

6 + 6 = 12

- 9. Write short notes about of the following:
  - (i) In vivo gene therapy with an example
  - (ii) Protein based diagnosis of corona virus
  - (iii) Production of peptide based recombinant vaccine against corona virus.

4 + 4 + 4 = 12

Department & Section	Submission Link
ВТ	https://classroom.google.com/c/MjM3NTQ0MzE0MTIz/a/MjkxNDM5MzQ2Mjg 2/details