



M.TECH DEGREE EXAMINATIONS: JUNE 2018

(Regulation 2015)

Second Semester

BIOTECHNOLOGY

P15BTT202 : Recombinant DNA Technology

COURSE OUTCOMES

- CO1:** Demonstrate the steps in cloning and expression of a foreign gene.
CO2: Explain and device various gene isolation methods from the selected organisms.
CO3: Describe expression and purification steps involved in recombinant protein Products.
CO4: Relate the application of GMOs in medical and Agriculture.
CO5: Understand issues related to release of GMO for commercial cultivation India.

Time: Three Hours

Maximum Marks: 100

Answer all the Questions:-

PART A (10 x 1 = 10 Marks)

1. The most commonly used vector for mammalian gene expression is CO1 [K2]
 - a) SV 40 based
 - b) Herps virus
 - c) Adeno virus
 - d) Caulimo virus
2. Which on one of the following is important for expression of foreign gene heterologous host? CO1 [K2]
 - a) Reporter gene
 - b) Signal peptide sequence
 - c) Ribosomal Binding Sequence
 - d) Lac operator
3. Positional cloning involves the following steps , arrange them in sequence CO2 [K₃]
 - i. Creating RFLP/RAPD marker linkage with phenotype of interest
 - ii. Construction of YAC library
 - iii. Screening of clones using marker sequence as probe
 - iv. Looking for ORF
 - v. Separation of chromosome by FACS
 - vi. Testing the activity ORF in mutant line for restoration of function
 - vii. Identification of location of marker and gene linked by FISH

7. Assertion (A) : Gene ligated to coat protein gene like pIII is expressed as fusing protein is display on the phage coat. CO4 [K4]
Reason (R) : Phage display technique is used to isolate the interacting proteins from a mixture.
- a) A and R are correct, R is the correct explanation for A b) A and R are correct, R is not the correct explanation for A
c) A is true R is false d) A is false and R is correct
8. What is the maximum number of alleles a SNP can have? CO4 [K₃]
- a) 4 b) 6
c) 12 d) 24
9. Assertion (A): Unwanted immunological reactions cannot be introduced by recombinant therapeutics in human body. CO5 [K₂]
Reason (R): The human body does not recognize them as foreign elements.
- a) A and R are correct, R is the correct explanation for A b) A and R are correct, R is not the correct explanation for A
c) A is true R is false d) A is false and R is correct
10. Conduct of recombinant DNA research in India is monitored by which one of the following agency at the centre? CO5 [K₃]
- a) GEAC b) RCGM
c) IBSC d) EPA

PART B (10 x 2 = 20 Marks)

11. What is the role of fusion tag in a recombinant protein? CO1 [K₃]
12. Why is *Saccharomyces cerevisiae* considered as better heterologous host for expressing eukaryotic proteins? CO1 [K₂]
13. Calculate the annealing temperature for the following forward and reverse primer set for a PCR . CO2 [K₃]
Forward primer: 5'ACTACGTACGTCAGTCAGTCAGCG3'
Reverse Primer: 5'GCGTCGTACGTCGATCGATCGATCGCG 3'
14. What is TA cloning? CO3 [K₃]
15. Draw the vector map of TOPO vector. CO4 [K₄]
16. Analyze the reasons for having regulated strong promoter for recombinant protein expression. CO3 [K₃]
17. State the role of a reporter gene. Give an example. CO4 [K₃]

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| 18. What is cis acting element? | CO4 | [K ₄] |
| 19. Relate the agencies and their role in GMO research and commercialization in India. | CO5 | [K ₂] |
| 20. List the recombinant proteins that are commercially used for treatment of human diseases. | CO5 | [K ₃] |

PART C (6 x 5 = 30 Marks)

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| 21. Draw the vector map of pET vector and critically analyze the role of each genetic element. | CO1 | [K ₂] |
| 22. How does dig-dUTP probe help to screen the library to isolate the gene of interest? | CO2 | [K ₃] |
| 23. Explain the steps involved in purification of a His-tagged recombinant protein using Ni ⁺ column. | CO3 | [K ₄] |
| 24. Discuss the principle of nanopore sequencing technique with its merits and demerits. | CO4 | [K ₃] |
| 25. Molecular techniques are useful in diagnosis of diseases' justify your statement with a techniques employed is it. | CO5 | [K ₂] |
| 26. Elaborate on the steps in Agrobacterium mediated DNA introduction into plant cell with sequence of steps and proteins involved. | CO1 | [K ₃] |

Answer any FOUR Questions

PART D (4 x 10 = 40 Marks)

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| 27. Explain the plant transformation vector – Binary vector and its use for developing a transgenic plant. | CO1 | [K ₃] |
| 28. Elaborate on the steps involved in construction of cDNA library from a tissue and screening the library using immunochemical method to get gene of interest? | CO2 | [K ₃] |
| 29. Analyze the factors that affect the successful expression of recombinant gene in heterologous system. | CO3 | [K ₃] |
| 30. Discuss the role of real time PCR in quantification of gene expression levels in various tissues and how is it useful in disease diagnosis? | CO4 | [K ₄] |
| 31. Explain the genes and enzymes introduced in metabolic engineering of rice for the expression of beta carotene? | CO5 | [K ₄] |
