



M.TECH DEGREE EXAMINATIONS: NOV /DEC 2024

(Regulation 2024)

First Semester

BIOTECHNOLOGY

24MBT502: Gene Expression and Analysis

COURSE OUTCOMES

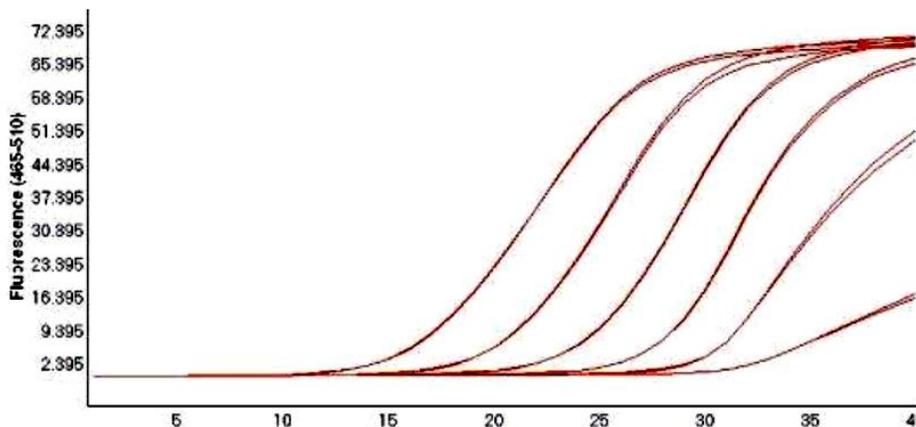
- CO1:** Apply knowledge of genetic manipulation in expression vectors to solve problems related to heterologous gene expression in prokaryotic systems.
- CO2:** Analyze methods for recombinant protein analysis to evaluate the effectiveness of various purification techniques.
- CO3:** Evaluate the impact of aberrant splicing and defective DNA methylation on the onset of genetic diseases by examining specific case studies.
- CO4:** Analyze the role of mitochondrial gene expression in cellular function to interpret its significance in disease pathogenesis.
- CO5:** Evaluate molecular diagnostic techniques, such as RT-PCR and qPCR, to assess their utility in identifying hospital-acquired infections and genetic disorders.

Time: Three Hours

Maximum Marks: 100

PART A (4*20 = 80 Marks)

1. The graph below represents the amplification curve obtained using RT-PCR, read the questions below and explain your answers with necessary schematic diagrams wherever applicable.



- a) Identify the C_t values of Samples with least and highest concentration of template DNA. 2 CO5 [K₃]
- b) How do you identify primer-dimers in RT-PCR reactions? 8 CO5 [K₂]
- c) Critically evaluate various strategies to be followed when designing primers to avoid non-specific amplification. 10 CO5 [K₃]
2. a) Draw the vector map of an expression vector and indicate the various genetic elements needed for successful expression of recombinant protein. 5 CO1 [K₃]
- b) Distinguish between Endpoint PCR and RT-PCR techniques 5 CO5 [K₃]
- c) Following datasets represents the restriction digestion results, create a restriction digestion map assuming that the template DNA is a plasmid named pGEN and Construct an agarose gel image by mapping the restriction fragments with a suitable ladder for the give table. 10 CO1 [K₄]

Restriction Enzyme	Size
EcoR I	20 kb
BamH I	2 kb, 6 kb, 12 kb
EcoR I + BamH I	2 kb , 4 kb , 6 kb, 8 kb

3. a) RNA interference (RNAi) is a technique used to silence a eukaryotic gene. Critically evaluate how RNAi can be performed in *Caenorhabditis elegans* and elaborate (with neat sketch) on the key genetic elements involved in RNA interference. 10 CO2 [K₃]
- b) Self-ligation of the vector DNA is a major problem in gene cloning. Critically evaluate the process involved in molecular cloning and explain how one can overcome the problem of self-ligation with suitable DNA manipulating strategy? 10 CO3 [K₄]
4. a) Elaborate on plasmid map of Ti Vector by analyzing the process of formation of crown gall disease in plants and explain the process of creating binary vectors that can be used to transfer gene of interest to plant genome. 10 CO1 [K₄]
- b) Elaborate on the procedure involved in detecting protein of interest using western blotting techniques and comment on the difference in detection probes used for northern and southern blots. 10 CO2 [K₂]

Answer any ONE Question

PART B (1*20 = 20 Marks)

5. a) Elaborate on role of mitochondrial gene expression and its possible genetic diseases in human. 5 CO4 [K₂]
- b) Design an experiment using SOE PCR to ligate a GFP gene (250 bp) with FimA gene (1000 bp) of *E.coli*. Explain your answers with a neat sketch wherever applicable and comment on appropriate controls required to ensure a gene specific amplification. 5 CO1 [K₃]
- c) Describe the causes and genetic factors behind Sickle cell anemia. In addition, present your answers with various diagnosis and treatment strategies for patients with early and advanced stages of sickle cell anemia 10 CO3 [K₃]

OR

6. a) Microfluidic (or) lab on chip devices offer unique advantages in both sorting and imaging of circulating tumor cells. The following questions are based on microfluidic devices-based diagnosis of cancer and provide your answers with neat sketch wherever applicable. 10+5 CO2 [K₂]
- i) Design an experiment using microfluidic device to mimic the blood flow in human capillary and to evaluate the usage of chemotherapeutic agent for patient care.
- ii) What are the merits and demerits of using microfluidic devices to mimic human capillary constrictions in studying CTC clusters?
- b) What is gene chip microarray? Elaborate on importance on Gene chip in disease diagnosis. 5 CO3 [K₃]
