UNIVERSITY OF JAFFNA, SRI LANKA

FACULTY OF ALLIED HEALTH SCIENCES

THIRD YEAR SECOND SEMESTER EXAMINATION IN BScHons (MLS) - 2023

MLSBM 3212 BIOTECHNOLOGY AND MOLECULAR BIOLOGY

Date: 02.06.2025 **Time: 1 Hour**

ANSWER ALL THREE QUESTIONS.

1.

1.1. Explain the role of the following components in DNA replication

1.1.1. DNA Topoisomerase **(10 Marks)**

1.1.2. DNA polymerase **(10 Marks)**

1.1.3. Origin binding protein **(10 Marks)**

1.2. Describe the major post-transcriptional modifications of pre-mRNA in eukaryotic cells. **(40 Marks)**

1.3. Describe the mechanisms of transcription termination in prokaryotes. **(30 Marks)**

2.

2.1. Write the principle of the reference method used for DNA extraction from pathological samples. **(40 Marks)**

2.2. List two methods used to assess the quality of extracted DNA **(10 Marks)**

2.3. Explain the working principles of,

2.3.1. Taq Man probes in real-time Polymerase Chain Reaction. **(25 Marks)**

2.3.2. Fluorescence In-situ Hybridization. **(25 Marks)**

3. Seven (07) blood samples of patients with suspected of hepatitis B infection and the necessary reagents are provided to you. You are requested to prepare a PCR master mix containing 1X PCR buffer, 1 mM $MgCl_2$, 0.1 mM dNTP mix, 0.5 µM of each primer, 1 Unit of Taq DNA polymerase, and 150 ng of template DNA in a total reaction volume of 20 µL.

The details of primers and other reagents are given below:

Primers for the *HBS* gene	Forward (5'->3'): TAGGACCCCTGCTCGTGTTA
	Reverse (5'->3'): CAGGAGTCGTGCAGGTTCTG
Product length	357 bp

PCR components	**Concentration**
Nuclease-free water	-
PCR buffer	10 X
$MgCl_2$	25 mM
dNTP	1 mM
Forward primer *HBS* gene	10 µM
Reverse primer *HBS* gene	10 µM
Taq DNA polymerase	5U/µL
Extracted genomic DNA of Hep B Virus	60 ng/µL

3.1. Calculate the volume of each PCR component required to prepare the master mix for the given samples. **(50 Marks)**

3.2. Calculate the melting temperature (Tm) and GC content of each primer pairs. **(20 Marks)**

3.3. Explain how endpoint detection of PCR products can be carried out in the above technique. **(30 Marks)**