



**PAMIBIA UNIVERSITY
OF SCIENCE AND TECHNOLOGY**

FACULTY OF HEALTH, APPLIED SCIENCES AND NATURAL RESOURCES

DEPARTMENT OF HEALTH SCIENCES

QUALIFICATION: BACHELOR OF MEDICAL LABORATORY SCIENCES	
QUALIFICATION CODE: 08BMLS	LEVEL: 6
COURSE CODE: MOD621S	COURSE NAME: MOLECULAR DIAGNOSTICS
SESSION: JANUARY 2023	PAPER: THEORY
DURATION: 3 HOURS	MARKS: 100

SUPPLEMENTARY/SECOND OPPORTUNITY EXAMINATION PAPER	
EXAMINER(S)	Ms. V. Tjijenda
MODERATOR:	Dr A Shiningavamwe

INSTRUCTIONS
<ol style="list-style-type: none">1. Answer ALL the questions.2. Write clearly and neatly.3. Number the answers clearly.

PERMISSIBLE MATERIALS

Scientific Calculator

THIS MEMORANDUM CONSISTS OF 5 PAGES (Including this front page)

SECTION A (10)

QUESTION 1

[10]

Evaluate the statements in each numbered section and indicate whether the statement is true or false. Write "true" or "false" next to the corresponding number and correct each false statement.

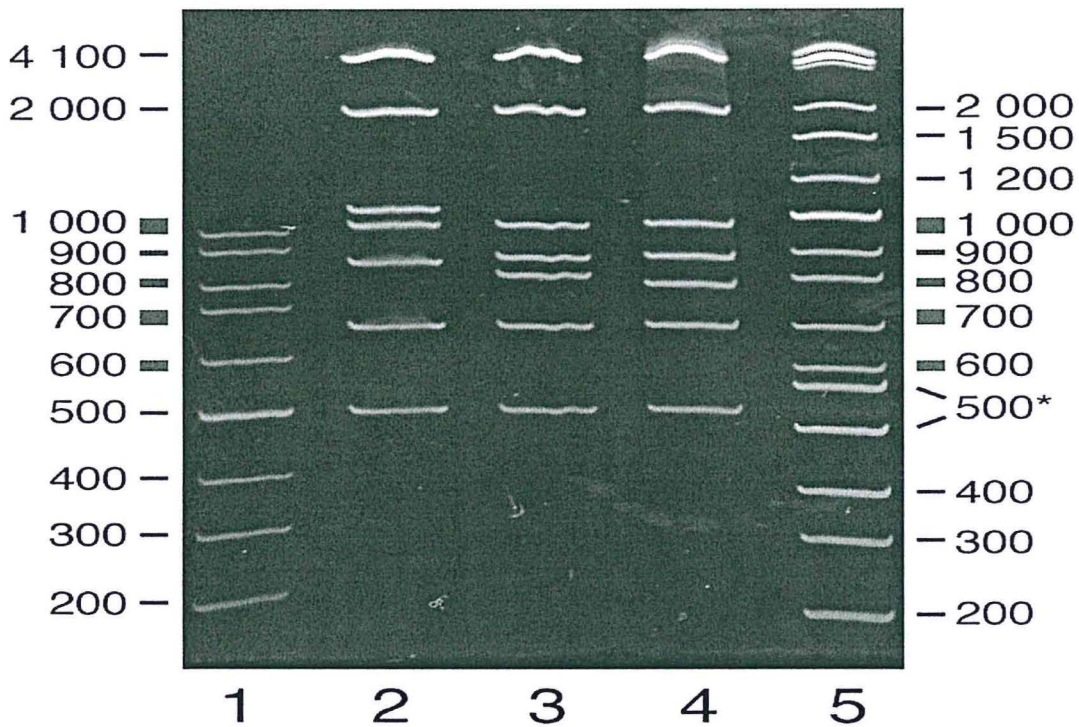
- 1.1 SYBR Green can bind to primer dimer including the desired target sequence during amplification.
- 1.2 T4 Polynucleotide Kinase is used for the addition of a hydroxyl group to an end having a free phosphate group.
- 1.3 The melting temperature of the CGGAGATTCTAGACCTCCTG is 66 °C.
- 1.4 During electrophoresis, high-molecular-weight DNA migrates slower than low-molecular-weight DNA through an agarose gel?
- 1.5 In preparing 250ml of a 0.8 % agarose gel, 0.8 g agarose is dissolved in 250ml TAE buffer.
- 1.6 Increasing the Mg²⁺ concentration in a PCR mixture increases the activity of Taq Polymerase and its specificity.

SECTION B (40)

QUESTION 2

[17]

- 2.1 When performing any conventional PCR it is important to include negative and positive controls. Explain what negative and positive controls are, and why is it necessary to include them. (6)
- 2.2 Study the image below and answer the questions that follow:



- 2.2.1 Identify the components of a master mix and their functions. (6)
- 2.2.2 Record the first 5 sizes of the DNA strands in lane 2. (From the top). (5)

QUESTION 3

[23]

- 3.1 During RNA extraction process, one of the solutions is DEPC-treated water. What is the benefit of using DEPC-treated water? (2)
- 3.2 Explain how UV spectrophotometry can be used to determine the DNA concentration and purity? (6)
- 3.3 Restriction enzymes recognize particular double-stranded DNA sequences and cut the backbone of both DNA strands near the sequence. Analyze the information below and answer the questions that follow. Consider the following small DNA sequence, target sequence (in bold) and restriction enzymes:

5' ATCG AATTCGG **GATCATTGCG** AATCCC 3'

3' TAGCTTAA **GGCCCTAG** **TAAGCGCTTAA** GGG 5'

Enzyme	Target sequence (cut at *) 5'→3'
EcoRI	G*AATTC
Bam HI	G*GATCC
Mbol	*GATC

- 3.3.1 Define palindromic sequence. (2)
- 3.3.2 For each of the restriction enzymes listed, give the number of times that the enzymes will cut the DNA fragment above. Also give the number of resulting DNA fragments after individual treatment with each enzyme. (6)
- 3.3.3 Calculate the T_m of the sense strand. (4)
- 3.3.4 Which enzyme will you use to clone the target sequence? (1)
- 3.3.5 Provide two reasons why you prefer the enzyme mention in **3.3.4** for plasmid cloning? (2)

SECTION C (50)

QUESTION 4

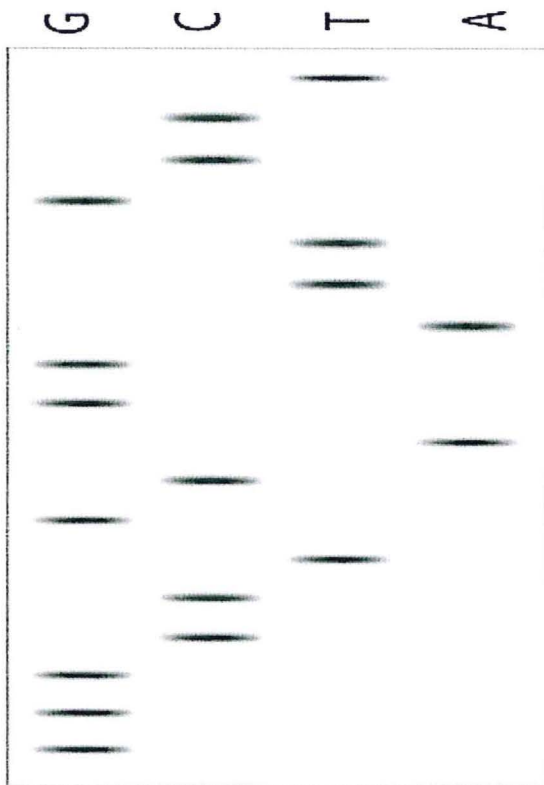
[20]

- 4.1 Describe four technical problems that can occur during the different steps in the Northern Blot Hybridisation method resulting in failure of a signal to appear on the final blot. (5)
- 4.2 Describe how the *FISH* technique is used to diagnose Philadelphia Chromosome. (7)
- 4.3 Discuss 'Nested Primer PCR' in detail. Under what circumstance would this type of PCR method be adopted and explain its benefit. (8)

QUESTION 5

[30]

- 5.1 Discuss the steps in Western Blotting technique. (10)
- 5.2 Summarize the main steps involved in setting up and running gel Electrophoresis. (10)
- 5.3.1 Define sequencing. (2)
- 5.3.2 Differentiate between Sanger sequencing and Maxam Gilbert. (4)
- 5.3.3 Below is the gel profile obtained from sequencing a gene using sanger sequencing. Provide the sequence of interest. (4)



END OF EXAMINATION!
