NAMIBIA UNIVERSITY
OF SCIENCE AND TECHNOLOGY

Faculty of Health, Natural Resources and Applied Sciences

School of Health Sciences

Department of Clinical Health Sciences

13 Jackson Kaujeua Street
Private Bag 13388
Windhoek
NAMIBIA

T: +264 61 207 2970
F: +264 61 207 9970
E: dchs@nust.na
W: www.nust.na

QUALIFICATION : **BACHELOR of MEDICAL LABORATORY SCIENCES**	
QUALIFICATION CODE: **08BMLS**	LEVEL: **6**
COURSE: **MOLECULAR DIAGNOSTICS**	COURSE CODE: **MOD 621S**
DATE: **NOVEMBER 2024**	SESSION: **1**
DURATION: **3 HOURS**	MARKS: **102**

FIRST OPPORTUNITY: EXAMINATION PAPER

EXAMINER: ***Ms Vanessa Tjijenda***

MODERATOR: ***Mrs Belinda Roselin Tsauses***

INSTRUCTIONS:

1. Answer all questions on the separate answer sheet.
2. Please write neatly and legibly.
3. Do not use the left side margin of the exam paper. This must be allowed for the examiner.
4. No books, notes and other additional aids are allowed.
5. Mark all answers clearly with their respective question numbers.

PERMISSIBLE MATERIALS:

1. Non-Programmable Calculator

ATTACHEMENTS

1. None

This paper consists of 7 pages including this front page

SECTION A: MULTIPLE CHOICE [10 MARKS]

QUESTION 1: [10 MARKS]

Evaluate the statements in each numbered section and select the most appropriate answer or phrase from the given possibilities. Fill in the appropriate letter next to the number of the correct statement/phrase on your ANSWER SHEET. [10]

1.1 The following enzyme is used to add ^{32}P to the 5' end of the DNA fragment. (1)

A. S1 Nuclease.
B. RNase H.
C. T4 Polynucleotide Kinase.
D. Terminal Deoxypolynucleotidyl Transferase.

1.2 Which molecule is detected during Southern blotting. (1)

A. Total RNA.
B. DNA.
C. Proteins.
D. mRNA.

1.3 Down Syndrome on chromosomes 21 found in children is associated with which chromosomal abnormality? (1)

A. Deletion.
B. Translocation.
C. Monosomy.
D. Trisomy.

1.4 Which assay is used to detect multiple gene resistance during microbiological analysis? (1)

A. Northern blotting.
B. Reverse transcriptase PCR.
C. Multiplex PCR.
D. Microarray.

1.5 The following is incorrect during nucleic acid extraction purity check. (1)

A. DNA 260/280 ratio of 2 is normal.
B. Phenol absorbs at 260 nm.
C. Proteins absorb at 260 nm.
D. RNA 260/230 ratio of 2.2 is normal.

1.6 Which of the following do not form part a PCR ingredient mixture? (1)

A. Forward and reverse primer.
B. All four ddNTPs.
C. cDNA.
D. Mg^{++}.

1.7 During Touch Down PCR (1)

A. The annealing temperature is manipulated over the course of the PCR.
B. Two primer sets are used during the PCR.
C. Antibodies are used to inactivate Taq Polymerase during lower temperature.
D. Normal conditions of a PCR are followed.

1.8 In preparing 250ml of 1x TAE buffer from 10x TAE stock solution, how much buffer is needed? (1)

A. 25 ml 10x TAE in 100 ml of double distilled water.
B. 1.25 ml 10x TAE in 250 ml of double distilled water.
C. 10 ml 10x TAE in 250 ml of double distilled water.
D. 10 ml 10x TAE in 100 ml of double distilled water.

1.9 During PCR, dNTPs are added during the _____ phase. (1)

A. Denaturation.
B. Extension.
C. Holding temperature.
D. Annealing.

1.10 The following method is useful in quantifying HIV viral copies: (1)

A. Real time PCR.
B. Variable Number Tandem Repeats.
C. Fluorescent in Situ Hybridization.
D. Sanger Sequencing.

SECTION B: SHORT QUESTIONS [72 MARKS]

Please answer ALL of the questions in this section.

QUESTION 2: [14]

2.1 During RNA extraction process, one of the solutions added is DEPC-treated water. Explain the benefit of using DEPC-treated water. (2)

2.2 Discuss in detail the steps involved in reverse transcription until before PCR. (5)

2.3 Identify the blotting technique used to identify mRNA? (1)

2.4 Briefly define "Multiplex PCR". (3)

2.5 Tabulate the differences between SYBR Green and Taq man probe in qPCR. (3)

QUESTION 3: [14]

Use the information proved below to answer the following question:

3.1 Shot gun sequencing is performed by identifying overlapping regions in the DNA sequences, aligning, the sequences and putting them back together to form the genome. Using the sequences below, construct the final sequence. (4)

GTTCCCACAGACC **ACCGTGTTTTCCGACCG**

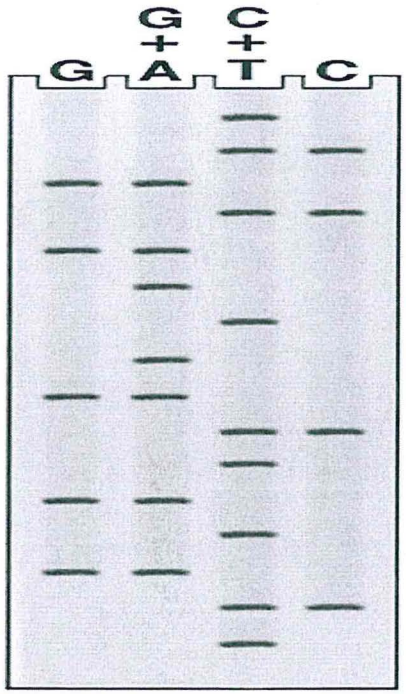
TCGATGCGGC

CGGCGAAGCATTGTTCC

3.2 The sequence in 3.1 contains an 18 bp long MecA gene found in drug resistant bacteria. Identify MecA gene by completing the following sequence.
5' TTGTTCCC... of the Gene. (4)

3.3 Was VNTR was used to sequence the first human genome? True/False. Justify your answer. (2)

3.4 The below image shows results obtained after using the chemical sequencing method. Provide the sequence of interest obtained after the sequencing method. (4)



QUESTION 4: **[21]**

4.0 Restriction enzymes recognize particular double-stranded DNA sequences and cut the backbone of both DNA strands near the sequence. Use the below information and answer the questions that follow. Consider the following small DNA sequence:

5' ATCG AATTCCG**G GATCATTCGCG** AATTCCC 3'

3' TAGCTTAA GGC**CCTAG TAAGCGC**TTAA GGG 5'

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

4.1 Define palindromic sequence. (2)

4.2 Calculate the Ta of the ant-sense strand. Show all your calculations. (3)

4.3 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. Present your answers in a table. (6)

4.4 Which enzyme will you use to clone the target sequence? (1)

4.5 Provide two reasons why you prefer the enzyme mentioned in 4.4 for plasmid. (2)

4.6 Describe how the FISH technique is used to diagnose Philadelphia Chromosome. (7)

QUESTION 5: **[23]**

Read the passage below and answer the questions that follows.

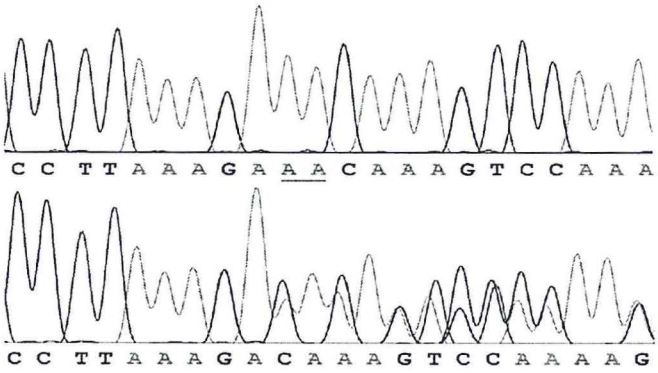
BRCA1 (BReast CAncer gene 1) and BRCA2 (BReast CAncer gene 2) are genes that produce proteins that help repair damaged DNA. Everyone has two copies of each of these genes—one copy inherited from each parent.

People who inherit a harmful change (also called a mutation or pathogenic variant) in one of these genes have increased risks of several cancers—most notably breast and ovarian cancer, but also several other types of cancer. People who have inherited a harmful change in BRCA1 *or* BRCA2 *also tend to develop cancer at younger ages than people who do not have such a variant.*

Nearly everyone who inherits a harmful change in the BRCA1 *or* BRCA2 *gene from one parent has a normal second copy of the gene inherited from the other parent. Having one normal copy of either gene is enough to protect cells from becoming cancer. But the normal copy can change or be lost during someone's lifetime. Such a change is called a somatic alteration. A cell with a somatic alteration in the only normal copy of one of these genes doesn't have sufficient DNA repair ability and can become cancer.*

A 32-year-old female with a family history of ovarian and breast cancer visited her doctor to find out whether she is at risk of developing breast cancer after recently losing her mother to breast cancer.

Below are the results obtained after sequencing BRCA1 gene:



BRCA 1 gene from non-carrier

BRCA 1 gene from the 32-year-old patient

5.1 Explain the principle of Sanger Sequencing. (2)

5.2 Interpret the mutation observed above. (2)

5.3 Using the information above, what advise will you give to the patient? (2)

5.4 What added value does sequencing have over annual breast examination and Pap smear? (2)

5.5 Generate the gel electrophoresis profile for the patient after Sanger Sequencing. (15)

SECTION C: LONG ANSWER QUESTIONS [20 MARKS]

Please answer ALL of the questions in this section.

QUESTION 6:

6.1 Explain the steps in Ion Torrent Sequencing. (10)

6.2 Discuss the steps involved in Western blotting. (10)

END OF EXAMINATION PAPER