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QUALIFICATION : **BACHELOR of MEDICAL LABORATORY SCIENCES**	
QUALIFICATION CODE: **07BMLS**	LEVEL: **6**
COURSE: **MOLECULAR DIAGNOSTICS**	COURSE CODE: **MOD621S**
DATE: **JANUARY 2024**	SESSION: **1**
DURATION: **3 HOURS**	MARKS: **100**

SECOND OPPORTUNITY / SUPPLEMENTARY: EXAMINATION QUESTION PAPER

EXAMINER: *Ms Vanessa Tjijenda*

MODERATOR: *Ms Cara Mia Dunaiski*

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. Genetic Code

This paper consists of 5 pages including this front page

SECTION A: TRUE / FALSE [10 MARKS]

QUESTION 1: TRUE/FALSE [10 MARKS]

Evaluate the statements and select whether the statement is true or false. Write the word 'True' or 'False' next to the corresponding number on your ANSWER SHEET.

1.1 The complete set of genetic information of a microorganism is called the genome. (1)

1.2 A hybridization method that uses DNA attached to a solid media to measure gene expression simultaneously is called Ion Torrent. (1)

1.3 Comparative hybridization technique can be used to compare gene expression in acute myeloid leukaemia and chronic myeloid Leukaemia. (1)

1.4 Fluorescent in situ Hybridization is used to diagnose Philadelphia Chromosome in leukaemia patients. (1)

1.5 Modified form of polymerase chain reaction (PCR) which avoids a non-specific amplification of DNA by inactivating the DNA polymerase at lower temperatures is called Hot Start. (1)

1.6 NGS technology that sequence DNA via three basic processes: amplify, sequencing and analyse using a bridging method is called pyrosequencing. (1)

1.7 Loading dye is used in gel electrophoresis to add weight to the nucleic acid and help with visualization under UV light. (1)

1.8 In preparing 250ml of a 0.8 % agarose gel, 0.8 g agarose is dissolved in 250ml TAE buffer (1)

1.9 Proteinase K inactivates nucleases. (1)

1.10 Short tandem repeats are found in the non-coding region of the DNA in each individual. (1)

SECTION B: SHORT ANSWER QUESTIONS [40 MARKS]

Please answer ALL of the questions in this section.

QUESTION 2 **(12)**

2.0 Sickle cell disease (SCD) is a genetic disorder caused by a mutation in both copies of a person's HBB gene. This gene encodes a component of haemoglobin, the oxygen-carrying protein in red blood cells. The mutation causes haemoglobin molecules to stick together, creating sickle-shaped red blood cells. This can lead to blood cell rupture, anemia, recurring pain, immunodeficiency, organ damage, and early death. Bone marrow transplants can cure SCD, but appropriate donors are hard to come by. The procedure also carries risks of dangerous side effects.

Below is the sequence for the normal haemoglobin molecule and the haemoglobin in SCD:
Normal mRNA sequence (HbA): 5' ACUCCUGAGGAG 3'
Mutated mRNA sequence (HbS): 5' ACUCCUGUGGAG 3'

2.1 Using the genetic code provided, write out the amino acids for HbA and HbS. (4)

2.2 Identify the type of mutation seen in the mutated mRNA. (1)

2.3 Why is sequencing better than RFLP to identify such a mutation. (2)

2.4 Describe the Sanger sequencing method. (5)

QUESTION 3 **[18]**

3.0 In a traditional PCR protocol, reaction components are assembled as described below. The final volume should be 50 µL.

1. Thaw all reagents on ice.
2. Assemble reaction mix into 50 µL volume in a thin walled 0.2 mL PCR tubes.
3. Add reagents in following order: water, buffer, dNTPs, Mg CL2, template primers, Taq polymerase.
4. Gently mix by tapping tube. Briefly centrifuge to settle tube contents.
5. Prepare negative control reaction without template DNA.
6. Prepare positive control reaction with template of known size and appropriate primers.

3.1 Identify the reagents that form part of a "master mix". (4)

3.2 Explain the addition of a negative and positive control and why they are prepared in that way. (4)

3.3 Explain important considerations when designing the primers. (10)

QUESTION 4: **[10]**

4.1 Define restriction enzyme. (1)

4.2 Design a 10 nucleotides palindrome sequence. (3)

4.2.1 Digest the palindrome sequence obtained in 4.2 such that it yields a blunt end. (2)

4.2.2 Digest the palindrome sequence obtained in 4.2 such that it yields a 3' sticky end. (2)

4.3 Provide the formula for calculating annealing temperature. (2)

SECTION B: LONG ANSWER QUESTIONS **[50 MARKS]**

Please answer ALL of the questions in this section.

QUESTION 5:

5.1 You are a masters' student at the Namibia University of Science and Technology. You are required to manually extract DNA from a bacteria culture for your study. Discuss how you would go about extracting the DNA using the manual chloroform/phenol protocol. Explain each step-in detail. (24)

5.2 Microarray is a technique used for gene expression profiling. Discuss in detail the principle of this technique and mention one advantage and one disadvantage of using this method. (10)

5.3 Nested PCR and Touch Down PCR are Conventional PCR method that can be modified to increase specificity. Explain how specificity is achieved in each method. (16)

END OF QUESTION PAPER

Second letter

First letter	U	C	A	G	Third letter
U	UUU Phe UUC Phe	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr	UGU Cys UGC Cys	U C
	UUA Leu UUG Leu		**UAA Stop** **UAG Stop**	**UGA Stop** UGG Trp	A G
C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His	CGU Arg CGC Arg CGA Arg CGG Arg	U C A G
			CAA Gln CAG Gln		
A	AUU Ile AUC Ile AUA Ile	ACU Thr ACC Thr ACA Thr ACG Thr	AAU Asn AAC Asn	AGU Ser AGC Ser	U C
	AUG Met		AAA Lys AAG Lys	AGA Arg AGG Arg	A G
G	GUU Val GUC Val GUA Val GUG Val	GCU Ala GCC Ala GCA Ala GCG Ala	GAU Asp GAC Asp	GGU Gly GGC Gly GGA Gly GGG Gly	U C A G
			GAA Glu GAG Glu		