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QUALIFICATION : **BACHELOR of MEDICAL LABORATORY SCIENCES**	
QUALIFICATION CODE: **07BMLS**	LEVEL: **6**
COURSE: **MOLECULAR DIAGNOSTICS**	COURSE CODE: **MOD 621S**
DATE: **NOVEMBER 2023**	SESSION: **1**
DURATION: **3 HOURS**	MARKS: **100**

FIRST OPPORTUNITY: 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. 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 How do you ensure that electrophoresis is running? (1)

A. By setting the correct voltage
B. By observing bubbles
C. By placing DNA closer to the negative electrode
D. Switch on the electrophoresis box

1.4 Which assay is used to measure multiple gene expression? (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 absorbs at 260 nm
D. RNA 260/230 ratio of 2.2 is normal

1.6 Calculate the annealing temperature of the following sequence: CGGAGATTCTAGACCTCCTG: (1)

A. 66 °C
B. 62 °C
C. 57 °C
D. 58 °C

1.7 Which reagent is used to detect DNA during PCR? (1)

A. SYBR Green
B. Loading dye
C. TAE
D. Ethidium bromide

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

A. 0.8 g
B. 2 g
C. 1 g
D. 0.9 g

1.9 During PCR, primers bind during the _____ phase. (1)

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

1.10 The following method is useful in identifying novel mutations: (1)

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

SECTION B: SHORT QUESTIONS **[60 MARKS]**

Please answer ALL of the questions in this section.

QUESTION 2: **[27]**

2.1 Define gel electrophoresis. (2)

2.2 Discuss the consideration when hooking up electrical current and running the gel (3)

2.3 Explain why we add loading dye to our DNA during gel electrophoresis? (2)

2.4 A postgraduate student is interested in determining the genes responsible for causing drugs resistance in bacteria (*Klebsiella pneumonia* and *Escherichia coli (E. coli)*). She runs a Multiplex PCR. The results are shown below:

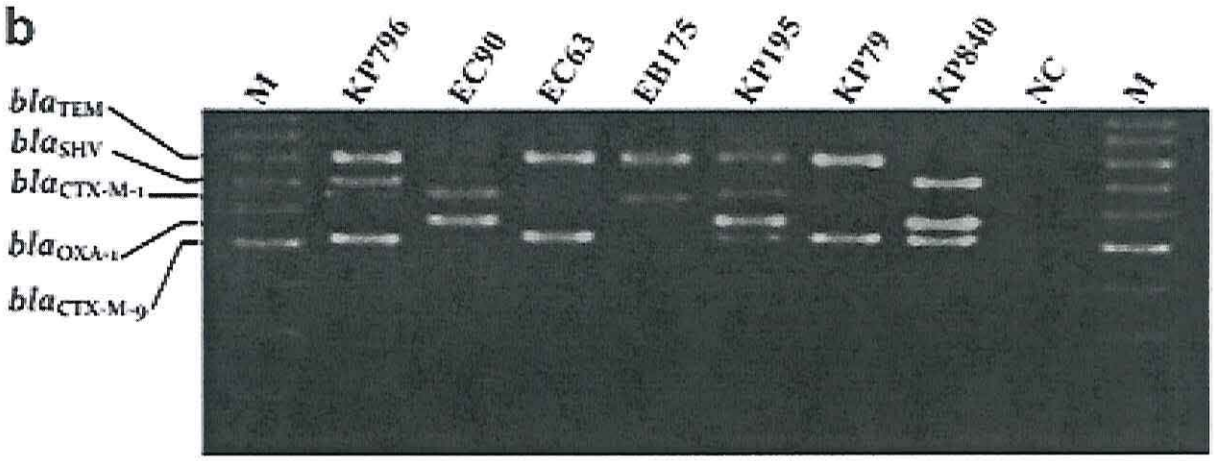

Figure 1: Lane M is the DNA ladder; **Lane 2** is a *Klebsiella pneumonia* strain KP796; **lane 3** is an *E coli* strain EC90; **Lane 4** is an *E coli* strain EC63; **Lane 5** as an *E coli* strain EB175; **lanes 6,7 & 8** are *Klebsiella pneumonia* strain KP195, KP79 and KP840 respectively. **Lane 9** is the negative control (NC). Bla- TEM, SHV, CTM-M1, OXA-1 and CTX-M9 are genes that are known to confer resistance in bacteria.

2.4.1 Briefly define "Multiplex PCR". (3)

2.4.2 Interpret the results for *E. coli* strain EC63 (land 4). (2)

2.4.3 Lane 9 contains the "Negative Control". Explain the purpose of adding a negative control to your PCR. (1)

2.5 List the ingredients used in a qPCR and briefly explain their purposes. (14)

QUESTION 3: **[12]**

Use the information proved below to answer the following question:

5' GAATTCTCGTACATAGGATCGATAGGCTAGACGAATTAGACTTACGTATAACGGGGTAGACAGA 3'
3' CTTAAGAGCATGTATCCTAGCTATCC GATCTGC TTAATCT GAATGCATATTGCCCCAT CTGTCT 5'

Primer 1: 5' TCGTACATAGGATCG 3'
Primer 2: 5' CTACCCCGTTATACG 3'

*Eco*RI recognition site: 5' GAATTC 3'
3' CTTAAG 5'

3.1 Using the DNA template shown above, underline the template sequence where the two primers will anneal. Label the underlined sequence "Primer 1" and Primer 2". (4)

3.2 Using the DNA template and the primers shown above, write the sequence of the resulting PCR product after 30 cycles. (4)

3.3 Define "restriction enzymes". (2)

3.4 Draw a box around the site(s) on the DNA that would be recognized and cleaved by *Eco*RI. (2)

QUESTION 4: **(10)**

4.1 Analysis of the spread and transmission of TB strains, using molecular methods, has been reported. Molecular techniques have been used in reliably differentiating *M. tuberculosis* isolates. Namibia has a high prevalence of HIV/AIDS, a cofactor for TB. The study done in Khomas region identified different TB strains circulating in the Khomas region. The most common strain was the **Beijing**, followed by the **Harlem strain** with the **LAM strain** being the least common. The below figure show some of the results.

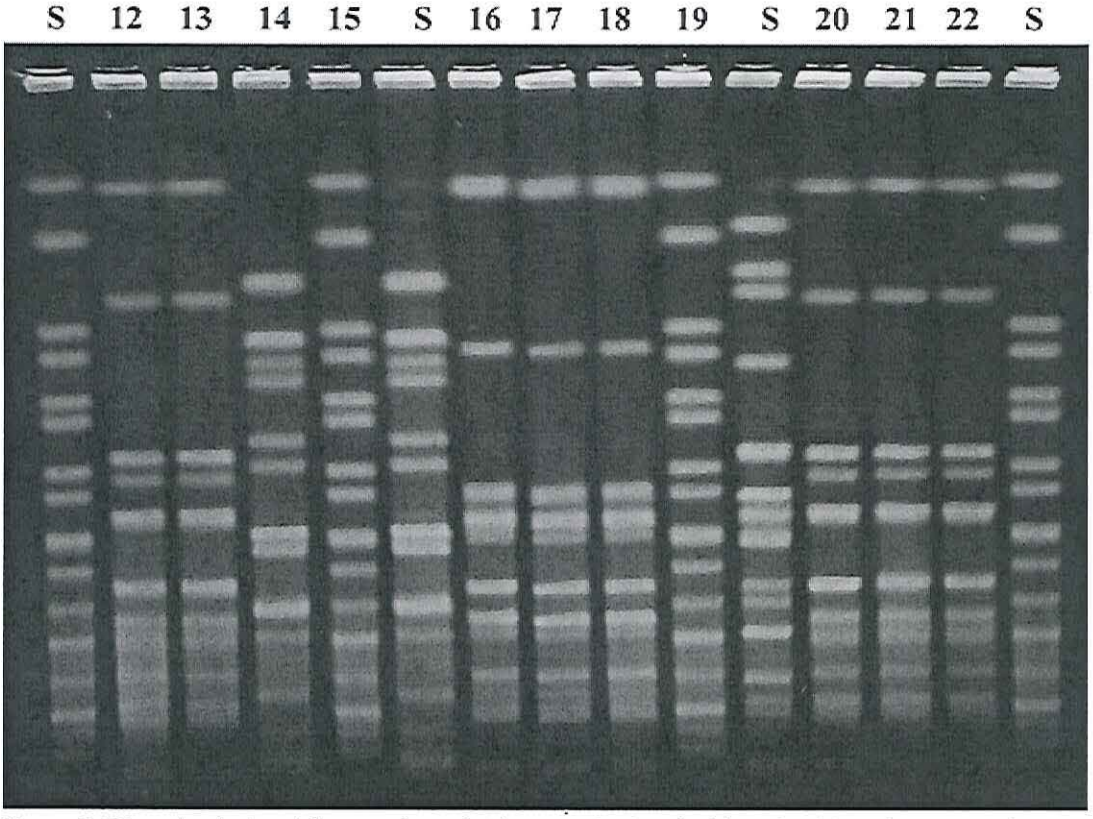

Figure 2: TB strains isolated from patients in Khomas. S = DNA ladder, 12 – 22 patient TB strains

4.1.1 Identify the method used. (1)

4.1.2 Use the results in **Figure 2** to corroborate the information provided above. (6)

4.2 Briefly discuss the principle of MALDI-TOF. (3)

QUESTION 5: **[10]**

5.1 Define DNA sequencing. (1)

5.2 Complete the following table by comparing the three assays. (9)

	Sanger Sequencing	Shot gun sequencing	Next Generation Sequencing
5.2.1 Principle			
5.2.2 length of DNA fragments sequenced			

SECTION C: LONG ANSWER QUESTIONS **[30 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 Northern blotting. (10)

6.3 Generate the gel electrophoresis profile of the following sequence using the Maxam Gilbert chemical method: **5' ACTGACTGAA 3'** (10)

END OF QUESTION PAPER