



**NAMIBIA UNIVERSITY  
OF SCIENCE AND TECHNOLOGY**

**FACULTY OF HEALTH, APPLIED SCIENCES AND NATURAL RESOURCES**

**DEPARTMENT OF NATURAL AND APPLIED SCIENCES**

<b>QUALIFICATION:</b> BACHELOR OF SCIENCE HONOURS	
<b>QUALIFICATION CODE:</b> 08BOSH	<b>LEVEL:</b> 8
<b>COURSE CODE:</b> MRT811S	<b>COURSE NAME:</b> METHODS IN RECOMBINANT DNA TECHNOLOGY
<b>SESSION:</b> JULY 2022	<b>PAPER:</b> THEORY
<b>DURATION:</b> 3 HOURS	<b>MARKS:</b> 100

<b>SECOND OPPORTUNITY QUESTION PAPER</b>	
<b>EXAMINER</b>	DR LAMECH MWAPAGHA
<b>MODERATOR</b>	DR RONNIE BOCK

<b>INSTRUCTIONS</b>
<ol style="list-style-type: none"><li>1. Answer ALL the questions.</li><li>2. Write clearly and neatly.</li><li>3. Number the answers clearly.</li><li>4. All written work <b>MUST</b> be done in <b>BLUE</b> or <b>BLACK</b> ink.</li></ol>

**PERMISSIBLE MATERIALS**

None

**THIS QUESTION PAPER CONSISTS OF FOUR (4) PAGES**  
(Including this front page)

**Question 1** [11]

- a) Discuss the mode of action of small interfering RNAs (siRNAs) (3)
- b) Messenger RNA caps are made in three steps, each performed by a different enzyme. Name the **THREE (3)** enzymes. (3)
- c) A Bachelor of Science research student would like to knock-out a gene of interest in a gene expression study using the CRISPR/Cas9 technique. Discuss in detail the mechanism behind this technique. (5)

**Question 2** [12]

- a) Briefly describe the Blue-white screening as a rapid and efficient technique for the identification of recombinant bacteria. (3)
- b) Describe **FOUR (4)** limitations of blue-white screening (4)
- c) ERA1, a gene identified in Arabidopsis, which is involved in Abscisic acid (ABA) signaling. ABA, a plant stress hormone, induces the closure of leaf stomata thereby reducing water loss through transpiration, and decreasing the rate of photosynthesis. Thus, plants lacking ERA1 activity have increased tolerance to drought. Discuss in details how one would go about cloning the ERA1 gene in a bacterial plasmid. (5)

**Question 3** [15]

- a) Give **SIX (6)** Similarities between the Ti and Ri Plasmids (6)
- b) Mention **THREE (3)** Limitations to the particle bombardment method, compared to Agrobacterium-mediated transformation (3)
- c) What are the steps followed in plant transformation using particle bombardment? (6)

**Question 4** [14]

- a) What are the functions of the following genes as utilised in genetic engineering? (3)
- I. Reporter gene;
  - II. Marker gene;
  - III. Selectable marker;

b) The Cre-Lox system relies on two components to function, a Cre recombinase and its recognition site loxP. Based on the orientation of the loxP sites, describe the **THREE (3)** outcomes that can result from the recombination. (6)

c) Describe the Cre Recombinase mechanism (5)

**Question 5** [18]

a) Explain the following medical applications of transgenic animals. (9)

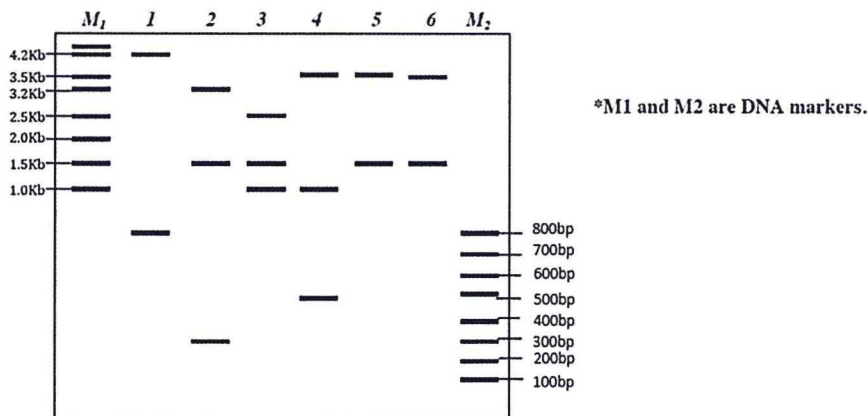
- I. Xenotransplanters;
- II. Tools in the study of immunoglobulin genes;
- III. Chemical Safety Testing;

b) There are several approaches in which scientific researchers may use in correcting faulty genes. Briefly describe **FOUR (4)** of these approaches (4)

c) Gene therapy is a medical approach that treats or prevents disease by correcting the underlying genetic problem. What are some of the risks envisioned in Gene Therapy? (5)

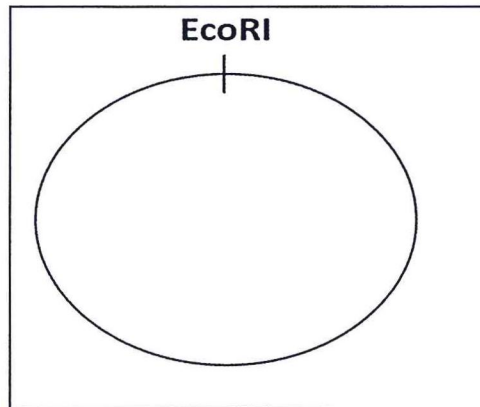
**Question 6** [12]

You are given a plasmid. In order to map this plasmid you set up a series of restriction digests and obtain the following results using agarose gel electrophoresis.



Lane	Digest	Size of fragments in bp
1	EcoRI and NotI	4200, 800
2	NotI and HindIII	3200, 1500, 300
3	HindIII and XbaI	2500, 1500, 1000
4	EcoRI and HindIII	3500, 1000, 500
5	HindIII	3500, 1500
6	XbaI and EcoRI	3500, 1500

- a) What is the approximate size of the plasmid? (2)
- b) Add the NotI, HindIII, XbaI restriction sites onto the plasmid map shown below. On your map give the distances between each of the restriction sites. (5)



- c) Mention **FIVE (5)** factors that make a Vector ideal for cloning. (5)

**Question 7** [18]

- a) The genetic code is the set of rules defining how the four-letter code of DNA is translated into amino acids, which are the building blocks of proteins. Discuss **THREE (3)** characteristics of the genetic code (6)
- b) Briefly describe the following mutations; (8)
- I. Nonsense Mutation;
  - II. Frame shift Mutation;
  - III. Silent Mutation;
  - IV. Missense Mutation;
- c) State **FOUR (4)** benefits of using mice in transgenic studies? (4)

**THE END**