CHUKA  **UNIVERSITY**

UNIVERSITY EXAMINATIONS

FOURTH YEAR EXAMINATION FOR THE AWARD OF DEGREE OF BACHELOR OF SCIENCE (BIOC)

BIOC 412: ADVANCED LABORATORY TECHNIQUES III

STREAMS: BSC (BIO) **TIME: 2 HOURS**

DAY/DATE: WEDNESDAY 11/4/2018 **11.30 A.M. – 1.30 P.M.**

INSTRUCTION: ANSWER ALLQUESTIONS IN SECTION A AND ANY OTHER TWO QUESTIONS IN SECTION B

1. The following is the DNA sequence of the wild type allele of Gene Z that you want to amplify using the polymerase chain reaction (PCR).

   If you amplify a DNA sequence through PCR what are the reaction components that you would absolutely need? Briefly state the function of each of these components.[5 marks]

2. (a) What is a plasmid? [1 mark]
   (b) List the qualities of a good vector. [4 marks]

3. Immunoassays are used to detect, quantify and characterize antigens. Briefly discuss the general procedure that underlie all immunoassays. [5 marks]

4. A 32year old man is found, during a private health screen, to have an aspartate aminotransferase (AST) activity 3.1 times higher than the local upper reference limit. Explain the rationale underlying this. [5 marks]

5. State the clinical application and analytical approaches commonly used to assay for urea. [5 marks]

6. World Health Organization guidelines for the diagnosis of diabetes (as adopted in the UK) recommend an oral glucose tolerance test if fasting plasma glucose concentration is in the range 6.1 – 6.9 mmol/L.

   (a) What is the diagnostic term applied to patients with fasting plasma glucose concentration in this range? [1 mark]

   (b) Why is an oral glucose tolerance test necessary in this group? [1 mark]

   (c) State the dose and composition of the glucose load used in the oral glucose tolerance test, indicating how it should be administered and the timing of the blood samples. [3 marks]

SECTION B: ANSWER ANY OTHER TWO QUESTIONS (20 MARKS)

1. (a) You have isolated two different yeast strains, strain 1 and strain 2, each of which fails to grow in the absence of arginine. You want to clone the wild type copy of the gene or genes that are mutated in strain 1 and strain 2. To do so, list the basic steps you will apply in gene cloning. [7 marks]

   (b) You choose the vector pBlue, show below. Note that the cloning site lies within lacZ, the coding region of the gene that encodes β-galactosidase. A cell that expresses β-galactosidase can take a substrate called X-gal and cleave the β-1, 6 linkage to form a product that is bright blue. For each of the following sequences found on pBlue, list the step or steps (1 -7 above) for which that sequence is needed and explain the role that sequence plays. [6 marks]

(c) You digest both the yeast genomic DNA and many copies of the vector with the BamH1 restriction enzyme. You mix the genomic fragments with the cut vectors and add DNA ligase. You then transform *E. coli* cells with the ligation mix and plate on solid agar medium. Describe what medium you could use to distinguish the bacterial colonies that carry a non-recombinant vector from the ones that carry a new recombinant vector. Explain how this media would allow you distinguish the bacterial colonies that carry a non-recombinant vector from the ones that carry a new recombinant plasmid. [4 marks]

(d) In the PCR reaction, you need a three step reaction cycle, which results in a chain reaction that produces an exponentially growing population of identical DNA molecules. Each step of a reaction cycle is performed at a specific temperature i.e. 95˚C for step 1, 55˚C for step 2 and 70˚C for step 3. Briefly explain why the three steps are performed under different temperatures. [3 marks]

2. Discuss the principle steps that underlie:

(i) The enzyme linkedimmunosorbent assay (ELISA) [10 marks]

(ii) Immunoblotting (Western Blotting) [10 marks]

3. Discuss enzymes which are most frequently used in clinical practice for diagnosis, prognosis and therapeutic monitoring of different liver pathologies. [20 marks]
