CHUKA **UNIVERSITY**

UNIVERSITY EXAMINATIONS

EXAMINATION FOR THE AWARD OF DEGREE OF DOCTOR OF PHILOSOPHY IN BOTANY

BOTA 931: PLANT BIOTECHNOLOGY

STREAMS: PhD BOTANY Y1S2   **TIME: 3 HOURS**

DAY/DATE: TUESDAY 04/12/2018   **2.30 P.M. – 5.30 P.M.**

INSTRUCTIONS:
- **Answer ANY THREE questions.**
- **Candidates are advised not to write on question paper.**
- **Candidates must hand in their answer booklets to the invigilators while in the examination room.**

QUESTION ONE (20 MARKS)

(a)   Explain the stages of plant tissue culture.   (5 marks)

(b)   Describe the cloning procedure using artificial plasmids as vectors.   (5 marks)

(c)   Discuss the methods of plant transformation.   (10 marks)

QUESTION TWO (20 MARKS)

(a)   Discuss the following methods of plant DNA purification in a molecular biology laboratory:

    (i)   Silica based column purification   (5 marks)

    (ii)   Dellaporta method   (5 marks)

(b)   Gel electrophoresis is routinely used in molecular biology and for analysis and preparation of DNA. Explain the principal behind agarose gel electrophoresis. (5 marks)

(c)   Polymerase chain reaction (PCR) amplifies a DNA template to produce multiple copies of a DNA fragment *in vitro*. Explain the PCR technique procedure. (5 marks)

QUESTION THREE (20 MARKS)

(a)   Describe DNA barcoding in plants using the ribulose-1, 5-bisphosphate carboxylase oxygenase large subunit gene (rbcL)   (10 marks)

(b)   Use examples to explain restriction enzyme digestion of purified PCR products.
                                                                                        (10 marks)

QUESTION FOUR (20 MARKS)

(a)   Discuss the principle of nanodrop spectrophotometry of nuclei acids and proteins based on absorbance and contaminant detection.   (10 marks)

(b)   Detail the methods of preparation of the following molecular analysis reagents.

   (i)   Chloroform: isoamyl alcohol (25:1)   (2 marks)

   (ii)   0.5 M EDTA, pH 8.0   (2 marks)

   (iii)   RNase A (20 mg/mL)   (2 marks)

   (iv)   20% SDS (sodium dodecyl sulphate)   (2 marks)

   (v)   Low salt TE-1 buffer   (2 marks)

QUESTION FIVE (20 MARKS)

(a)   The following sequence data was observed on CLC main workbench software. Describe possible causes of the observed variation.   (5 marks)

(b)   Compare and contrast homology and comparative genome based approaches of sequence data analysis.   (5 marks)

(c)   Explain the following methods of phylogenetic analysis of molecular data:

      (i)   Maximum parsimony   (5 marks)

      (ii)   Maximum Likelihood   (5 marks)

      (iii)   Bayesian Test   (5 marks)