

UNIVERSITY OF RUHUNA

BACHELOR OF SCIENCE (GENERAL DEGREE) LEVEL III (SEMESTER I)
EXAMINATION – AUGUST 2017

SUBJECT: BOTANY

COURSE UNIT: BOT3182 (Advanced Molecular Biology)

Time: 1 1/2 hrs

Answer three questions including question No.1 and 2

Index No:

1.

a) Underline the correct answer

- i). The polymerase chain reaction is important because it allows us to
- a) insert eukaryotic genes into prokaryotic plasmids.
 - b) make DNA from RNA transcripts.
 - c) make many copies of a targeted segment of DNA.
 - d) insert regulatory sequences into eukaryotic genes.
 - e) B and C
- ii) The enzyme reverse transcriptase enables scientists to produce.
- a) Restriction endonucleases
 - b) cDNA molecules
 - c) Restriction fragment length polymorphisms
 - d) Blunt end restriction fragments
 - e) mRNA transcripts
- iii) Where is the messenger RNA synthesized in a eukaryotic cell?
- a) nucleus
 - b) nucleolous
 - c) ribosome
 - d) cytoplasm
 - e) golgi body
- iv) Bacteria containing recombinant plasmids are often identified by,
- a) examining the cells with an electron microscope
 - b) using radioactive tracers to locate the plasmids
 - c) exposing the bacteria to an antibiotic that kills cells lacking the plasmid
 - d) removing the DNA of all cells in a culture to see which cells have plasmids
 - e) producing antibodies specific for each bacterium containing a recombinant plasmid

v) Bacteria that contain pBR322 plasmid with a foreign DNA fragment in the tetracycline gene, would grow

- a) in the broth containing tetracycline, but not in the broth containing ampicillin.
- b) in the nutrient broth plus tetracycline
- c) in the nutrient broth plus ampicillin, but not in the broth containing tetracycline.
- d) only in the broth containing both antibiotics.
- e) in all four types of broth.

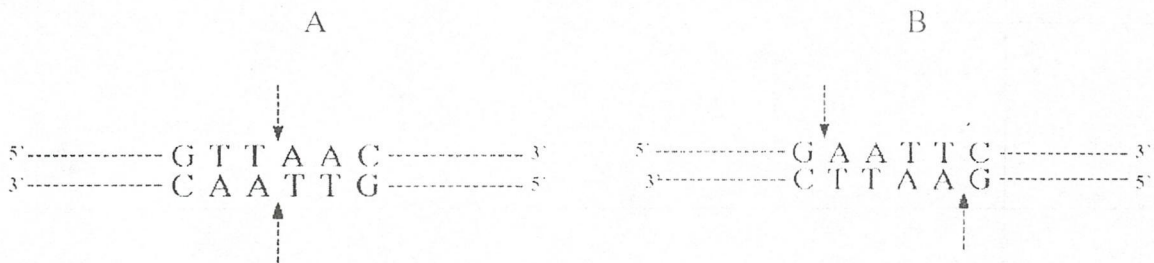
(10 marks)

b) List the five factors to be considered when selecting a plant material to extract DNA.

(25 marks)

c) What are the properties of different types of restriction enzymes? Giving reasons mention what type of restriction enzyme is used in genetic engineering? **(20 Marks)**

d) Recognition base sequences of restriction enzymes A and B are as below.



Indicate the products after a single restriction digestion of each sequence. **(20 marks)**

e) Mention 5 different enzymes used in molecular biology? What are the roles of them?
(25 marks)

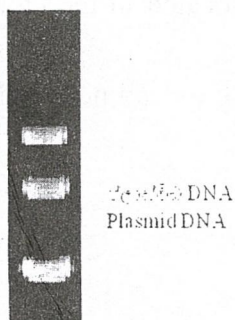
Enzyme	Role
1	
2	
3	

4	
5	

2.

a) What is the relationship between agarose concentration and the size of the DNA fragments to be separated by electrophoresis? **(10 marks)**

b) Following figure shows the banding pattern of a plasmid DNA sample after electrophoresis. How would you explain the results? **(10 marks)**



c) How do you denature a DNA molecule?

(12 marks)

d) 'Denaturation and renaturation are very important properties of the DNA molecule'. State the practical importance of these properties of DNA in Molecular Biology? (6 marks)

e) 'DNA cross-linking is an essential step in DNA blotting'. Give reasons. (7 marks)

f) You have a clone of 1.5 kb DNA fragment inserted into a *BamH* I restriction site in a 2 kb molecular weight plasmid vector. There are two *Pst* I sites located in the cloned fragment and one *Kpn* I site is present near the *BamH* I site of the vector.

What is the size (in kb) of the recombinant molecule? Draw a line diagram to show the restriction sites of the recombinant molecule. (15 marks)

g) Display the expected restriction digestion pattern on an agarose gel after treatment with *Kpn* I enzyme.

(10 marks)

h) How many DNA fragments do you expect if you double digest the recombinant molecule with *Pst* I and *Kpn* I? Explain your results using suitable diagrams only.

(20 marks)

3.

a) What are the key features of a cloning vector? Indicate how they are important in cloning? (20 marks)

b) Complete the following table (20 marks)

Vector type	Characteristic features	Size of the DNA fragment that can be inserted (kb)

c) Using **diagrams only**, illustrate a method to clone and select bacterial cells transformed with a reporter marker gene from non-transformed cells. **(6 x10 =60 marks)**

4.

a) List the steps of 'Southern blotting'. **(20 marks)**

b) Give the facts that should be considered in each step in this technique. **(10 marks)**

c) Mention 2 detection methods used in 'Southern blotting'. **(2 marks)**

d) Mention the 4 different types of primers used in a PCR **(8 marks)**

e) Briefly explain the steps in a PCR **(30 marks)**

f) Fill in the following table giving examples **(30 marks)**

Probe type	Advantages	Disadvantages
1.		
2.		