

RAJARATA UNIVERSITY OF SRI LANKA FACULTY OF APPLIED SCIENCES, MIHINTALE

B.Sc. (Special) Degree in Applied Biology

Fourth Year Semester I Examination – April/ May 2015

MIB 4203 – TECHNIQUES AND STRATEGIES OF MOLECULAR BIOLOGY

Time: Two (02) hours

Answer ALL questions.

- 1. a) Describe Define the following using no more than a single sentence for each
 - i. Southern blot
 - ii. Northern blot
 - iii. Western blot

(30 Marks)

b) Describe the functions of molecules involved in detection of a specific DNA sequence by biotinilation. Use the following diagram as a guide. (35 Marks)

Tetramethylbenzidine TMB

TMB

TMB

H2O2

HRP

SA

Biotinylated D17Z1 probe

Immobilized question DNA

Membrane

c) Figure 1 shows the absorption spectra of chlorophyll a and chlorophyll b.

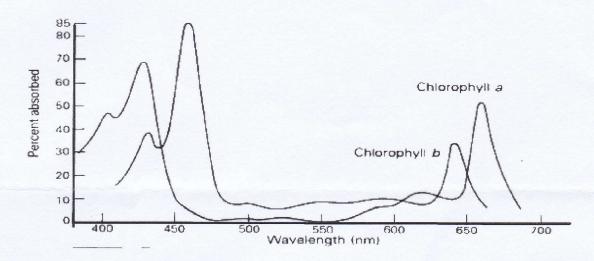


Figure 1. Absorption spectra of chlorophyll a and chlorophyll b

i. Assume that you are required to assess the level of contamination of isolated **chlorophyll** a with **chlorophyll b.** Propose a strategy based on the above spectra and provide reasons for your choice. (25 Marks)

ii. Although the proposed approach for question 1(c)i may be used to assess the contamination of **chlorophyll b** with **chlorophyll a**, it will not be as sensitive as for the above (Chlorophyll a with chlorophyll b). Mention the reason for this. (10 Marks)

2. a) Compare and contrast BACS and YACS.

(50 Marks)

b) Describe how posttranscriptional gene silencing (PTGS) be achieved by siRNA.

(50 Marks)

- 3. a) Describe the modified yeast two hybrid assay for membrane proteins and justify the need for modification. (40 Marks)
 - b) A group of scientists claims that protein **A** exists in two forms, the unphosphorylated and phosphorylated. While the unphosphorylated form is found in the cytoplasm, the phosphorylated form is found only in the nucleus. Signal **X** perceived by receptor **Y** causes the autophosphorylation of **Y** and subsequent transfer of the phosphate group to **A**. If the DNA sequences of both X and the auto kinase involved is known, design a method to prove or disprove the claim. (60 Marks)

- 4. a) With the help of labeled diagrams, illustrate tandem affinity assay and appraise its importance compared to other protein purification methods. (40 Marks)
 - b) Briefly describe the principle behind MALDI-TOF

(30 Marks)

c) Critically analyze **three** ionization techniques of your choice in terms of importance in protein sequencing. (30 Marks)

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