RAJARATA UNIVERSITY OF SRI LANKA
FACULTY OF APPLIED SCIENCES

Bachelor of Science Honours in Microbiology

Fourth Year - Semester I Examination – July/ August 2023

MIB4205 – TECHNIQUES AND STRATEGIES OF MOLECULAR BIOLOGY

Index No..... **Time: Two (02) hours**

Answer ALL questions.

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1. Select the most appropriate response and underline. Answer **ALL parts** [a) to n)] **(100 marks)**

a) Two methods that can be used to estimate the DNA concentration and purity of a DNA extract are
   i. Spectrophotometry and Fluorimetry
   ii. Gel electrophoresis and Dot blot
   iii. Spectrophotometry and Gel electrophoresis
   iv. Fluorimetry and Dot blot

b) Phenol which is used in DNA extraction, precipitates
   i. DNA and leaves proteins in the aqueous solution.
   ii. RNA-protein complexes and leaves DNA in the aqueous solution.
   iii. cell debris and leaves nucleic acid-protein complexes in the aqueous solution.
   iv. proteins and leaves nucleic acids in the aqueous solution.

c) When random amplification is needed
   i. short primers and high annealing temperatures can be used.
   ii. long primers and high annealing temperature can be used.
   iii. short primers and low annealing temperature can be used.
   iv. long primers and low annealing temperature can be used.

d) Select the matching pair from the following.
   i. Isothermal PCR – Strand displacement
   ii. RT-PCR – Inverse PCR
   iii. High GC primer – Low annealing temperature
   iv. Hot start PCR – Law precise amplification

e) Select the correct words from i. to iv. below to fill the gaps in the following sentence.
   In the shotgun approach of DNA sequencing _____ sequences are cloned and sequenced from _____ of cloned DNA.
   i. random, one end
   ii. random, both ends
   iii. specific, both ends
   iv. specific, one end

f) The results of pyrosequencing is interpreted by 36.

i. comparing the relative masses of nucleotides present in the DNA.
ii. comparing relative amounts of enzyme tags associated with each nucleotide.
iii. looking at captured light emissions that correspond with the addition of a nucleotide.
iv. breaking down nucleotides and analyzing the byproducts.

g) Select the correct response from i. to iv. below to fill the gaps in the following sentence.

_____ is a platform that can be used in library preparation by both emulsion and polony PCR.

i. Semiconductor sequencing (Ion Torrent)
ii. Pyrosequencing – 45
iii. Reversible Terminator Sequencing (Illumina)
iv. Sequencing by Ligation (SOLiD)

h) Epigenetic modifications can be detected only by

i. Sanger Sequencing
ii. Shotgun sequencing
iii. Second generation sequencing
iv. Next generation sequencing

j) RITS- RNA Induced Transcriptional Silencing operates by

i. inhibiting RNA polymerase and hence preventing transcription completely.
ii. binding to DNA template strand and hence blocking the transcription.
iii. modifying histones and thereby inducing heterochromatin formation.
iv. modifying histone and thereby inducing euchromatin formation.

k) Both siRNA and miRNA

i. are generated by exogenous dsRNA.
ii. are generated endogenously in the nucleus.
iii. are ssRNA molecules.
iv. have endonuclease activity.

l) miRNA mediates post-transcriptional modification by

i. destabilization of mRNA.
ii. inhibiting translation.
iii. both destabilization of mRNA and translational inhibition.
iv. heterochromatin formation.

m) Select the correct combination of statements on *in vitro* mutagenesis.

i. The mutations can be done at will. Phenotypic screening of variants is always necessary.
ii. The mutations can be done at will. Random mutagenesis is not possible.
iii. Hot spots lead to nonrandom mutagenesis. Directed multiple changes are possible.
iv. Directed multiple changes are possible. Variants can be screened both phenotypically and genotypically.

n) Directed mutagenesis in the control region of a gene may lead to
   i. change of amino acid sequence of the peptide.
   ii. construction of hybrid proteins.
   iii. change in expression of a peptide.
   iv. production of peptide having altered characteristics.

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2. **Answer <u>ALL</u> parts (a – f) in the space provided.** **(100 marks)**

a) How does salt cause precipitation of DNA in DNA extraction? **(10 marks)**

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b) If you have sequences of both genomic and cDNA sequences of a human gene, how would you determine the intron sequences of the gene? **(15 marks)**

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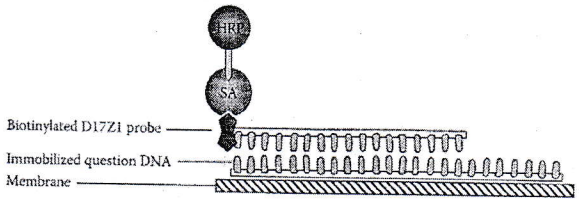
c) The following aschematic diagram shows use of slot blot for detecting a specific sequence of DNA. **(25 marks)**



State the functions of

i. Membrane .....

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ii. Probe .....

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iii. Biotinylation .....

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iv. Streptavidin .....

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iv. HRP .....

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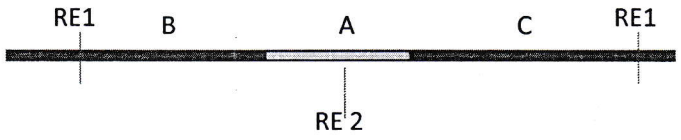
d) The following is a diagram of a sequence of DNA which has a segment of which the base sequence is known (A) and two segments of which the base sequence is unknown (B and C). RE are restriction sites, which generate overhanging sequence.

Explain the process of PCR amplification to amplify segments B and C limited by restriction sites, **using a fully labeled schematic diagram only.** **(30 marks)**



e) Why is Sanger Sequencing considered a chain termination method of DNA sequencing? **(10 marks)**

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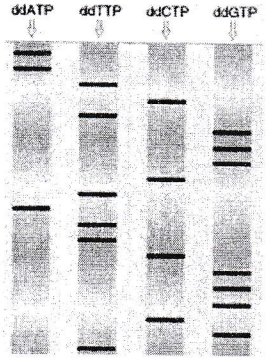
f) Using the following chromatogram of Sanger Sequencing, deduce the first 10 bases of the DNA fragment, starting from 5' end. **(10 marks)**



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3. a) Explain **two (02)** natural functions of RNA interference (RNAi). **(40 marks)**

b) RNAi is a useful technology in gene silencing and regulation of gene expression. In this technology, a sequence of DNA that codes for RNA with a hairpin loop is engineered. A plant virus requires expression of its coat protein for further invasion in the infected plant.

Using the above information, propose a mechanism to genetically engineer a resistant plant for this virus. **(60 marks)**

4. Write short notes on the following. **(100 marks)**

a) qPCR –Technique and Applications

b) TaqMan Technology

c) Megaprimer Method of Site Directed Mutagenesis

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