RAJARATA UNIVERSITY OF SRI LANKA
FACULTY OF APPLIED SCIENCES

B.Sc. (Special) Degree in Applied Biology

Fourth Year Semester II Examination – February/March 2019

MIB 4206 – MOLECULAR BIOTECHNOLOGY

Time: Two (02) hours

Answer **ALL** questions.

1. Hen's egg ovalbumin is a well-known egg white allergen causing immunological hypersensitivity (IgE-mediated) in children suffering from egg allergy. Ovalbumin is known as a potent allergen that triggers IgE-mediated immune response (that means IgE binds to ovalbumin). To develop an in vitro ELISA based diagnostic test for diagnosis of egg allergy a principle investigator of a researche laboratory has chosen ovalbumin as the antigen of choice (or allergen in this case). However, the principle investigator prefers to use recombinant ovalbumin rather than commercially available natural ovalbumin extract. Recombinant ovalbumin will be used to exhibit the level of IgE-binding in patients sera using the ELISA technique to demonstrate the presence of anti-ovalbumin IgE antibodies. The principle investigator has decided to express recombinant ovalbumin in an *E. coli* protein expression system for the first time.

   a. When analyzing the IgE-reactivity of recombinant ovalbumin using patients' sera would you recommend using control experiment (s)? Provide reasons for your decision. **(15 marks)**

   b. Principle investigator has decided to use recombinant ovalbumin instead of natural ovalbumin for in vitro diagnosis of egg allergy. Is this a good choice? Provide your opinion on this. **(15 marks)**

   c. What sort of challenges will the researcher face when producing recombinant ovalbumin in an *E. coli* protein expression system? Provide strategies to overcome the challenges you mentioned. **(70 marks)**

2. Discuss the fundamental concept of nucleic acid diagnostic systems and their advantages over traditional diagnostic methods (e.g. clinical diagnosis, immunology-based methods etc.). Provide a brief example on how it can be used to diagnose an infectious disease. **(100 marks)**

3. You discover that a thermostable and rapid acting cellulase is expressed by a wild strain of *Clostridium thermocellum* that you isolated from a hot spring. *Clostridium thermocellum* is a slow-growing organism and you would like to clone and over express this cellulase gene in a different organism to purify substantial quantities of this enzyme for further study.

Assume that the full DNA sequence of the cellulase is known and the PCR primers are already available. You have the following at your disposal;

DNA extraction kit, PCR primers (forward and revere – containing restriction sites for BamHI and HindIII) for *C. thermocellum* cellulase, cloning vector pT7T3-18U (also containing restriction sites for BamHI and HindIII), expression vector pET28a (containing restriction sites BamHI, HindIII, Kanamycin resistance marker gene and a poly-histidine tag in the expressed protein), *E.coli* BL21 competent cells, growth media, Kanamycin, IPTG, X-gal, restriction enzymes BamHI and HindIII, DNA ligase, cell lysis buffer, Nickel affinity chromatography column with buffers, an imidazole solution and common molecular biology reagents and equipment.

a. Write an account of the workflow that you are likely to follow, in order to overexpress the thermostable cellulase in *E. coli* host (use a flow-diagram if required). **(70 marks)**

b. Justify the rationale behind using an imidazole solution to elute the expressed protein from the Nickel affinity column. **(30 marks)**

4. Write short notes on the following sequencing technologies

a. Sanger (Dideoxy nucleotide) sequencing
b. Illumina sequencing
c. Ion-Torrent sequencing
d. Nanopore Min-Ion sequencing **(100 marks)**

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