3936700 SIMI/M : MAJOR: MICROBIOLOGY; M.Sc. (MICROBIOLOGY)
KEY WORDS : DENSONUCLEOSIS / *Aedes albopictus* DENSOVIRUS (*Aal* DNV) / INSECT PARVOVIRUS
KUSAVADEE EK-U: CONSTRUCTION OF FULL-LENGTH DNA CLONES OF DENSOVIRUS. THESIS ADVISORS: SA-NGA PATTANAKITSAKUL, D.Med.Sc., PRIDA MALASIT, MD, F.R.C.P., 167 P. ISBN 974-664-666-4.

Densovirus is a small single-stranded DNA, non-enveloped virus that belongs to the subfamily *Densovirinae* of the *Parvoviridae* family. This group of viruses are invertebrate viruses that infect exclusively insects. Two of the major densoviruses, the *Aedes aegypti* (*Aae* DNV) and *Aedes albopictus* (*Aal* DNV) densoviruses infected mosquitoes that were known to carry viruses responsible for two important public health diseases, namely, dengue hemorrhagic fever and, yellow fever. The understanding of these viruses and their interactions with the disease viruses are therefore very important and may lead to a possibility of using the viruses as either biological vectors or as a means to introducing foreign genes into the indigenous mosquitoes.

This project involves the cloning and molecular characterization of a densovirus found infecting C6/36 cell line obtained from the Department of Epidemiology and Public Health, Yale University (Dr. Pattamaporn Kittayapong). The construction of the full-length clone was from the assembly of clones from the direct cloning of restriction enzyme digestion and PCR amplifications of genomic DNA obtained from the virion DNA collected from the supernatant of C6/36 infected cells. Three major fragments were obtained and assembled into the full-length genomic clone. The genomic clone had been sequenced and the sequence was found to be similar to *Aedes albopictus* (*Aal* ) DNV as described earlier in C6/36 cells by Boublik *et al.* 1994, who described a virus found contaminated in the laboratory C6/36 cell line. It is therefore likely that the virus cloned by this study might also be contaminated in the cell line used. The sequence of the newly cloned virus was 98% homologous to the virus described. In order to characterize whether the full-length DNA clone is infectious, the above mentioned construct was transfected into C6/36 cells by using lipofectin technique. The full-length clone had been shown to be non-infectious, i.e. it could not produce live infectious virions as demonstrated by repeated culture of the cell supernatants into naïve uninfected cells. *In situ* hybridization by FITC-labeled densovirus specific peptide nucleic acid (PNA) failed to demonstrate any newly synthesized cytoplasmic densovirus DNA. Molecular characterization of the full-length clone found a 44-bp deletion at the left terminal region and extra 53-bp insertion in the right terminal region. 15 substitutions and 3 insertions along the genome were found to be different from the reported *Aal* DNV. It was concluded that the deletion/insertion found in the newly cloned DNA were major interfering factors, which is the reason why this clone failed to be infectious.