Thesis Title	Cloning of Dengue Virus Envelope-protein Gene Fragments and Expression of Peptide Domains as Fusion Proteins in *Escherichia coli*
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ABSTRACT

Dengue virus antigen preparation for use in immunological methods such as hemagglutination inhibition (HI), enzyme linked immunosorbent assay (ELISA), and dot enzyme immunoassay (DEIA) usually relies on time-consuming and laborious procedures of suckling mouse brain inoculation or mosquito-cell culture. An alternative method of viral antigen preparation using recombinant DNA technology is being researched. Previously, the dengue virus gene encoding envelope (E) protein has been cloned into a plasmid vector, pGEX-3X, to express as glutathione-S transferase (GST)-E fusion protein in *E. coli* but it was found to be insoluble and unable to purify by affinity chromatography. This work reports an ongoing study of cloning fragments of the dengue virus E-protein gene by aiming to produce soluble fusion proteins. Cloning of the

E-protein gene fragments encoding its four peptide domains (A1, C, A2, and B) into pGEX-3X vector resulted in insoluble GST-fusion proteins although two fusion proteins (GST-A1 and GST-B) were reactive to antibodies in pooled convalescent sera (PCS). Further fractionation of more reactive B peptide domain produced loss of reactivity of this peptide domain. Cloning of the E-protein gene fragment encoding the B peptide domain from all four serotypes of dengue viruses into pMAL-c2 vector to express as maltose-binding protein (MBP)-B fusion proteins gave rise to soluble proteins that could be purified by affinity chromatography using amylose resin. All MBP-B fusion proteins were reactive to antibodies in PCS as tested by Western blot method and DEIA but only MBP-B from dengue 2 virus was reactive to monoclonal antibody-3H5, confirming that 3H5 is specific to its epitope in the E protein of dengue 2 virus. The MBP protein alone was also found to bind to antibody in normal sera and PCS. The attempt to cleave and remove MBP from the MBP-B fusion protein was unsuccessful. The interference of MBP in antibody detection made the fusion proteins produced less likely to be used in development of immuno-diagnostic test. A new expression system not dependent on fusion with a carrier protein such as that expresses a required protein with six-histidine tag for purification with nickel- or cobalt-resin column is suggested.