Thesis Title Some Approaches For the Molecular Cloning of *Plasmodium vivax* DNA in *Escherichia coli*

Name Miss Manee Pookanjanatavip

Degree Master of Science (Biochemistry)

Thesis Supervisory Committee

Associate Professor Prapon Wilairat, Ph.D.

Associate Professor Sakol Panyim, Ph.D.

Professor Yongyuth Yuthavong, D.Phil.

Date of Graduation April 29, 1988

ABSTRACT

P. vivax-infected blood samples (20-50 ml per case) were made available from patients who visited the outpatient clinic of the Malaria Eradication Center of the Ministry of Public Health. The average parasitemia and white cell count was 0.17% and 0.13% respectively. Passage of the blood samples through a CF-11 cellulose column failed to remove white cells, and only resulted in loss of half of packed red cell volume.

Three approaches were taken for the construction of *P. vivax* DNA library. Firstly, the less dense band of *P.*

vivax DNA, obtained by Hoechst dye 33258-cesium chloride density centrifugation, was digested with Sau3AI and ligated with BamHI-digested pUC12 vector. Secondly, Sau3AI-digested P. vivax DNA reannealed to sonicated human DNA was ligated with BamHI-digested pUC12 vector. Thirdly, EcoRI*-digested P. vivax DNA reannealed to sonicated human DNA was ligated with EcoRI-digested pUN121 vector. These ligated products were used to transform E. coli JM 107 cells by the DMSO method. 5,000 clones that were obtained from all libraries were first screened by colony hybridization, and potential P. vivax specific clones were then selected. The specificity of these clones were confirmed by Southern or dot-blot hybridization.

However, despite a great deal of effort, generation of P. vivax-specific DNA probes was unsuccessful. This may suggest that there was too little P. vivax DNA material present in the starting preparation, or that there were considerable homology between repetitive DNA sequences of P. vivax and the human genome.