Thesis Title Construction of DNA Probe for the Detection of *Plasmodium vivax*

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ABSTRACT

Approximately 45% of malaria cases in Thailand are caused by *Plasmodium vivax*. Unlike *P.falciparum*, *in vitro* culture of *P.vivax* has not been successful. Previous molecular cloning of *P.vivax* specific DNA probe had failed due to human DNA contamination. In this study, DNA isolated from *P.vivax*-enriched patients blood was used to construct a genomic library. DNA was digested with Sau3A I and ligated to Bluescribe M13+ vector at BamH I site. Approximately 4,000 recombinant clones were screened for repetitive DNA using *P.vivax* DNA as the probe. From 23 clones containing repetitive DNA, 7 clones did not hybridize with human DNA. When these DNA clones were further hybridized with *P.falciparum* DNA followed by *P.vivax* DNA, 3 clones gave positive signal with *P.vivax* DNA probe. One of these clones, designated B2 contained 2 vectors and 2 inserted DNA. The 1.1 Kb inserted DNA fragment of B2 could detect *P.vivax* DNA to the level of 6.25 ng and did not hybridize to human , *P.falciparum*,

P.chabaudi, *P.berghei*, *P.cynomolgi* and mosquito DNA. This *P.vivax* specific fragment was subcloned into Bluescribe M13+ vector and named pMU-PV2. The nucleotide sequence of pMU-PV2 was determined. It contained 1,161 bp with 47.5% G+C content, with several short internal repeating sequences, runs of 5-7 consecutive A and T and no significant peptide and splice site sequences.

Five primer pairs for amplification of *P.vivax* infected blood were designed based upon the above *P.vivax* specific sequence. The amplification condition was optimized for sensitivity of the detection. One of these primer pairs which allowed amplification of 183 bp sequence of *P.vivax* DNA exhibited *P.vivax* specificity. Moreover, the sensitivity of 183 bp detection in *P.vivax*-infected blood was at 10^{-6}% parasitemia, which is equivalent to 1 parasite in 20 ul blood.

For the field validity, 233 field samples were tested and evaluated. The specificity and sensitivity of PCR method comparing to microscopic examination were 70.8 and 75.6%, respectively, with 25.3% disagreement.