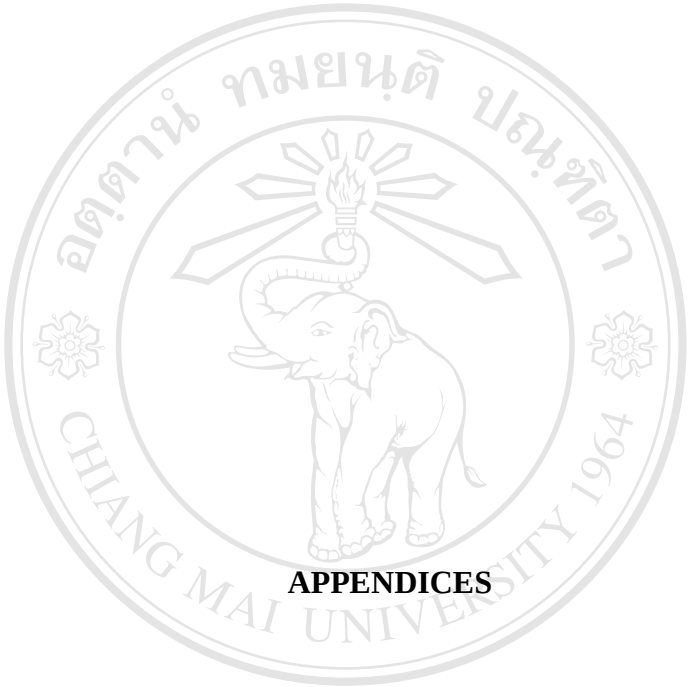

APPENDICES

APPENDIX A

LIST OF CHEMICALS AND REAGENTS USED IN THIS STUDY

Chemicals	Sources
1 kb plus DNA ladder	Fermentas Life Sciences, USA
5-Fluorocytosine	Sigma-Aldrich, USA
Acetic acid	Lab Scan, USA
Agarose powder	USB, USA
Ampicillin	TP Drug Laboratory
Artesunate	Sigma-Aldrich, USA
Adenosine triphosphate (ATP)	Fermentas Life Sciences, USA
Bovine serum albumin	Sigma-Aldrich, USA
Bromphenol blue	USB, USA
Calcium chloride ($CaCl_2$)	Merck, Germany
CF11 cellulose powder	Whatman, USA
Chloroform	Merck, Germany
Chloroquine	Sigma-Aldrich, USA
D-glucose	Merck, Germany

Chemicals	**Sources**
Diethyl pyrocarbonate (DEPC)	Ambion, USA
Dimethyl sulfoxide (DMSO)	Merck, Germany
DNase I	Boeringer Manheim
dNTPs	Fermentas Life Sciences, USA
Ethanol	Scharlau, South Australia
Ethidium bromide	Sigma-Aldrich, USA
Ethylenediaminetetraacetic acid (EDTA)	Fluka, USA
Giemsa solution	Merck, Germany
Glycerol	Carlo Erba, USA
Heparin	Sigma-Aldrich, USA
HEPES	Sigma-Aldrich, USA
Hoechst 33258	Sigma-Aldrich, USA
Human T-cell solution	Amaxa GmbH, Switzerland
Hydrochloric acid (HCl)	Merck, Germany
Isoamyl alcohol	Merck, Germany
Isopropanol	Merck, Germany
Magnesium chloride ($MgCl_2$)	Fluka, USA
Magnesium sulfate ($MgSO_4$)	Merck, Germany

Chemicals	**Sources**
Methanol	Merck, Germany
Neomycin sulfate	Invitrogen, USA
Nycodenz powder	Sigma-Aldrich, USA
Paraformaldehyde	Merck, Germany
Pfu polymerase	Promega, USA
Phenol	Pierce, USA
Phenylhydrazine-HCl	Sigma-Aldrich, USA
Potassium acetate (CH_3COOK)	Merck, Germany
Potassium chloride (KCl)	Carlo Erba, USA
Potassium dihydrogen phosphate (KH_2PO_4)	Carlo Erba, USA
Potassium hydrogen carbonate ($KHCO_3$)	Carlo Erba, USA
Potassium hydroxide (KOH)	Merck, Germany
Peptone, Bacto	Difco Laboratories
Proteinase K	Invitrogen, USA
Pyrimethamine	Sigma-Aldrich, USA
Restriction enzymes	NEB, USA
RNase A	Sigma-Aldrich, USA
RPMI1640	Gibco, USA

Chemicals	**Sources**
Saponin	Sigma-Aldrich, USA
Sodium acetate (CH_3COONa)	Merck, Gemany
Sodium chloride (NaCl)	Carlo Erba, USA
Sodium citrate	Merck, Germany
Sodium dodecyl sulfate (SDS)	USB, USA
Sodium hydrogen carbonate ($NaHCO_3$)	Carlo Erba, USA
Sodium hydrogen phosphate (Na_2HPO_4)	Carlo Erba, USA
Sodium hydroxide (NaOH)	Carlo Erba, USA
T4 DNA ligase	Promega, USA
Taq polymerase	Promega, USA
Tris	Pacific Science, USA
Triton X-100	Sigma-Aldrich, USA
Trizol	Sigma-Aldrich, USA
Tryptone	Criterion, USA
Tween 20	USB, USA
Whatman 3MM	Whatman, USA
Yeast extract	Criterion, USA

APPENDIX B

LIST OF INSTRUMENTS USED IN THE STUDY

Instrument	Company
1.5-ml microcentrifuge tube	Sorensen, USA
1 and 10-ml sterile syringe	Scientific Industries, USA
175 cm^2 cell culture flask	CORNING, USA
37^oC incubator	Heraeus Holding GmbH
50-ml centrifuge tube	CORNING, USA
96-well culture plate	CORNING, USA
Amaxa Nucleofector machine	LONZA, Switzerland
Amaxa Nucleofector Kit	LONZA, Switzerland
Automatic pipette	Gilson, USA
Balance	Ohaus Scout Pro, USA
Balance	Precisa Gravimetrics AG, USA
Balance CP3233	Sartorius AG, USA
Block Heater SBH130D	Stuart®, USA
Centrifuge	Hettich Holding GmbH, USA

Instrument	**Company**
Centrifuge 5417R	Eppendorf, USA
Centrifuge 5810R	Eppendorf, USA
ClassII Biological Safety Cabinets	NUAIRE, USA
CO_2 tank	TSG Gas Services
Cover glasses	Menzel-Glaser, USA
Cryotube	Thermo Fisher, USA
Deionized water machine	Thermo Fisher, USA
Distilled water machine	Hamilton Laboratory
DNeasy Blood&Tissue Kit	QIAGEN, USA
Duran sterile bottle	Duran Group GmbH
ELISA plate, flat bottom 96 wells	Thermo Fisher, USA
FACS CYTOMICS FC500 MPL	Beckman Coulter
Filter set up	Sartorious AG, USA
Flat bottom well plate, 96 well sterile	CORNING, USA
Fluorescence microscope	OLYMPUS
Gas mixture (5%CO_2, 1%O_2 and 94%N_2)	TSG Gas Services
Gel electrophoresis machine	Cosmo Bio Co., Ltd.
Gel/PCR DNA Fragment Extraction Kit	Geneaid, Taiwan

Instrument	**Company**
Genomic DNA Mini Kit (Blood/Culture cell)	Geneaid, Taiwan
Glass slide	HBG Henneberg-Sander
Heat lamp	Scientific Products
Heat-Stir (Stirrer Hotplate)	Stuart®, USA
High-Speed Plasmid Mini Kit	Geneaid, Taiwan
Hybond N^+ Nylon Membrane	GE Healthcare, USA
Insulin syringe	Becton Dickinson
Laminar flow	Labline, USA
Light microscope	OLYMPUS
Liquid N_2 tank	Taylor-Wharton
Microcentrifuge for 1.5-ml tube	LabScientific, USA
Microcentrifuge for PCR tube	LabScientific, USA
Microscopic slide	Sail Brand
MinElute PCR/Gel Extraction Kit	QIAGEN, USA
Nanodrop ND1000	Nanodrop Technologies, USA
Needle no. 21 and 27	Scientific Industries
Orbital Shaker	Barnstead Lab-Line, USA
Paraflim	Pechiney Plastic Package

Instrument	**Company**
Petri-dish, plastic 90 mm	Sterilin®, USA
pH meter B200	Eutech Cybermetics
Pipette Boy	Stripettor Costar
Pipette tips	Sorensen, Bioscience
QIAprep Spin Miniprep Kit	QIAGEN, USA
QIAquick Gel Extraction Kit	QIAGEN, USA
QIAquick PCR Purification Kit	QIAGEN, USA
Refrigerator	Sharp
Refrigerator -20^{o}C	Brandt
Refrigerator -80^{o}C	Forma Scientific
Refrigerator -80^{o}C	REVCO
Speed Vac System	Thermo Savant
UV crosslinker	Hoefer Scientific Instruments, USA
Vortex	Scientific Industries
Water bath	Memmert
Water bath	Labline, USA
Water bath shaker G76D	Brunswick Scientific
X-ray film	Godak

APPENDIX C

PREPARATION OF REAGENTS AND BUFFERS USED IN THIS STUDY

1. LB (Luria Beroom temperatureani) culture medium/ agar

In order to prepare LB culture medium, 1% of tryptone, 0.5% of yeast extract and 1% of NaCl are dissolved in DW and autoclaved at 120^{o}C for 15 min. For preparation of LB agar, 1.5% of agar is added prior to autoclave. The medium is stored at room temperature.

2. Preparation of Ampicillin (100 mg/ml)

Five grams of ampicillin sodium salt is dissolved completely in 50 ml of DW and filter sterilized through a 0.22 µm filter. Finally, the suspension is aliquoted into 1.5-ml microcentrifuge tube (1 ml/tube) and stored at -20^{o}C.

3. Preparation of LB containing ampicillin

Ampicillin solution (100 mg/ml) is added into LB medium or LB agar (The agar is melted completely with microwave oven) at a final concentration of 100 µg/ml.

4. P1 suspension buffer for plasmid extraction

This suspension buffer is prepared by adding 10 ml of 1M Tris-HCl (pH 8.0) and 4 ml of 0.5M EDTA (pH 8.0). DW is subsequently added up to 200 ml and the mixture is autoclaved at 120^{o}C for 15 min. The buffer is stored at 4^{o}C.

5. P2 lysis buffer for plasmid extraction

The lysis buffer is prepared by adding 20 ml of 2M NaOH and 20 ml of 10% (v/v) SDS. DW is then added up to 200 ml and stored at room temperature.

6. P3 neutralizing buffer for plasmid extraction

Sixty milliliters of 5M CH_3COOK is combined with 23 ml of 100% acetic acid. DW is finally added up to 200 ml and stored at 4^{o}C.

7. TAE electrophoresis buffer

Tris (387.6 g) is dissolved in 3 L of DW. After that, 160 ml of 0.5M EDTA (pH 8.0) and 91.5 ml of 100% acetic acid are added. The final volume is adjusted up to 8 L with DW and stored at room temperature.

8. TE buffer

The buffer is performed by combining 10 ml of 1M Tris-HCl (pH 8.0) with 2 ml of 0.5 M EDTA (pH 8.0). DW is added to adjust the volume up to 1 L and autoclaved for 15 min at 120^{o}C. TE buffer is kept at 4^{o}C.

9. Glycerol stock of bacteria

Escherichia coli can be stored for many years at -80^{o}C in medium containing 15% glycerol. 0.15 ml of glycerol (100%) is added to a 2-ml screw-cap vial. Then, 0.85 ml of bacterial overnight culture in LB medium containing ampicillin is added. The glycerol stock is kept at -80^{o}C.

10. 10X Phosphate buffer saline (PBS)

The components of 10X PBS (stock solution) contain 0.01M KH2PO4, 1.37M NaCl and 0.027M KCl pH 7.0. For working solution, 10 X stock solution is diluted 10 times with DW and pH is then adjusted to 7.2 with 1M HCl. The solution is sterilized by autoclaving for 20 min at 120^{o}C.

11. *Plasmodium berghei* freezing solution

Thirty percent of glycerol (v/v) is prepared in 1x PBS, autoclaved at 120^{o}C for 15 min and stored at 4^{o}C.

12. Heparin stock solution

Heparin powder is dissolved in DW to a concentration of 25,000 units/ml, filters sterilized (0.2 µm) and stored at 4^{o}C. For working solution, 0.2 ml of heparin stock solution is added with 25 ml of RPMI1640 culture medium without FBS in order to create a final solution of 200 units/ml, and stored at 4^{o}C.

13. Phenylhydrazine stock solution

250 mg of phenylhydrazine powder is dissolved completely in 10 ml of 0.9% NaCl and stored at -20^{o}C.

14. RPMI1640 culture medium

RPMI1640 powder (with L-glutamine and 25 mM HEPES, without $NaHCO_3$) is dissolved in 1 L of DW. 0.85 g of $NaHCO_3$ and 5 ml of neomycin sulfate stock solution are added. The mixture is filter sterilized (0.2 µm) and stored at -20^{o}C in 100 ml aliquots.

15. Complete culture medium

RPMI1640 culture medium is supplemented with FBS to a final concentration of 30% (v/v).

16. Neomycin sulfate stock solution

10 mg/ml of neomycin sulfate stock solution is prepared in DW.

17. Nycodenz stock solution

138 g of Nycodenz powder is dissolved in 500 ml of Nycodenz buffer (density of 1.15 g/ml at 20^{o}C), sterilized by autoclaving for 20 min at 120^{o}C, and stored at 4^{o}C.

18. Nycodenz buffer

5 mM Tris-HCl, 3 mM KCl, 0.3 mM $CaNa_2EDTA$ are mixed, adjusted to pH 7.5 and stored at 4^{o}C.

19. Pyrimethamine solution in drinking water

Pyrimethamine is dissolved in DMSO to a final concentration of 7 mg/ml (stock solution). The stock solution is then diluted 100 times with tap water and pH is adjusted to 3.5-5.0 using 1M HCl.

20. Hoechst33258 stock solution

Hoechst33258 powder is dissolved in DW to a final concentration of 500 µM and stored at -20^{o}C.

21. Erythrocyte lysis buffer stock solution

1.5M NH_4Cl, 0.1M $KHCO_3$, and 0.01M Na_2EDTA are mixed, adjusted to pH 7.4 and stored at room temperature. For a working solution, stock solution is diluted 10 times with DW and stored at 4°C.

22. Parasite lysis buffer

10 mM Tris pH 8.0, 0.4 M NaCl, 1 mM EDTA, and 1% SDS are mixed and stored at 4°C.

23. 0.15% Saponin

0.15 g of saponin powder is dissolved in 100 ml of RPMI1640 culture medium, filter sterilized and stored at 4°C.

24. 10X Maleic acid buffer

58 g of Maleic acid and 43.8 g of NaCl are dissolved in 500 ml of DW. pH is the adjusted to 7.5 with NaOH and autoclaved. For 1X Maleic acid buffer, the 10X buffer is diluted 10 times with DW and stored at room temperature.

25. Washing buffer for Southern blot

1X Maleic acid buffer is mixed with 0.3% Tween 20 (v/v) and stored at room temperature.

26. 10X Blocking solution

Blocking reagent 10% (w/v) (bottle 4 of DIG kit) is dissolved in 1X Maleic acid buffer at 65°C with placing on a stirrer for mixing. The solution is autoclaved

and stored at 4^{o}C. For 1X Blocking solution, 10X Blocking solution is diluted with 1X Maleic acid and stored at 4^{o}C.

27. Detection buffer

Detection buffer contains 0.1M Tris-HCl and 0.1M NaCl, pH 9.5.

CURRICULUM VITAE

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Education	
March, 1995	Certificate of junior high school Piboonbumpen Demonstration School, Chon Buri
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Scholarship	Thailand Graduate Institute of Science and Technology (TGIST) 2005-2007

Training Experience

1. Functional Genomic of Malaria Parasites, October 9-21, 2005 at International Center for Genetic Engineering and Biotechnology (ICGEB), New Delhi, India
2. Functional Genomic of Malaria Parasites, March 18-26, 2007 at National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand Science Park, Pathumthani, Thailand
3. Laboratory animal experiment and care, July 6-7, 2010 at National Laboratory Animal Center, Mahidol University, Thailand

Poster Presentations

1. Keystone Symposia on Molecular and Cellular Biology: Pathogenesis and Control of Emerging Infectious and Drug-Resistant Organisms (S4), October 22-27, 2008 at Royal Orchid Sheraton Hotel, Bangkok, Thailand
2. Vivax Malaria Research III: 2009 and Beyond, May 24-28, 2009 at Gamboa Rainforest Resort, Panama
3. Research Advances in Malaria Conference at John Hopkins University, Baltimore, Maryland, May 28-29, 2009
4. Keystone Symposia on Molecular and Cellular Biology: Malaria-New Approaches to Understanding Host-Parasite Interactions (F1), April 11-16, 2010 at Copper Mountain, Colorado, United States
5. 2010 The XIIth International Congress of Parasitology (ICOPA): Understanding the Global Impact of Parasite-from genomics to function and

disease, August 15-20, 2010 at The Melbourne Exhibition and Convention Center (MECC), Melbourne, Australia

Paper Publications

1. **Somsak V**, Srichairatanakool S, Kamchonwongpaisan S, Yuthavong Y and Uthaipibull C. Small-scale *in vitro* culture and purification of *Plasmodium berghei* for transfection experiment. *Mol Biochem Parasitol* 177 (2011) 156-159
2. **Somsak V**, Uthaipibull C, Prommana P, Srichairatanakool S, Yuthavong Y and Kamchonwongpaisan S. Transgenic *Plasmodium* parasites stably expressing *Plasmodium vivax* dihydrofolate reductase-thymidylate synthase as *in vitro* and *in vivo* models for antifolate screening. Malaria Journal (2011)

Paper Submission

1. **Somsak V**, Srichairatanakool S, Yuthavong Y, Kamchonwongpaisan S and Uthaipibull C. Flow cytometric enumeration of *Plasmodium berghei*-infected red blood cells stained with SYBR Green I. *Acta Tropica* (2011)