APPENDICES

APPENDIX A

LIST OF THE CHEMICALS AND INSTRUMENTS

1. Chemicals

All chemicals used as in this study were analytical grade reagents.

Chemical name	Source
4', 6-diamidio-2-phenylindole (DAPI)	Molecular Probes, Eugene, OR, USA
Acrylamide	Biorad, Hercules, CA, USA
Agarose (electrophoresis grade)	Sigma-Aldrich. St.Louis, MO, USA
Amersham Hybond^{TM}-ECL	GE healthcare Bio-Sciences Co. Piscataway, NJ, USA
Ampiillin	Sigma-Aldrich. St.Louis, MO, USA
BCA Protein Assay	Thermo Fisher Scientific Inc., Rockford, IL, USA
BIACORE 2000™ biosensor	Biacore AB, Uppsala, Sweden
Bis-acrylamide	Biorad, Hercules, CA, USA
Bovine Serum Albumin (BSA)	Sigma-Aldrich. St.Louis, MO, USA
B-PER II Bacterial Protein Extraction Reagent	Pierce, Rockford, IL, USA
Bromphenol blue	Sigma-Aldrich. St.Louis, MO, USA

Chemical name	Source
COBAS® AMPLICOR HIV-1 Monitor Test, v1.5	Roche Molecular Systems, Inc., Branchburg, NJ, USA
DTT	AMRESCO, Salon, OH, USA
Dulbecco's Modified Eagle's medium (DMEM)	Gibco, Grand Island, NY, USA
ECL system	GE Healthcare, Buckinghamshire, UK
EDTA	Sigma-Aldrich. St.Louis, MO, USA
EMSA kit [E33075]	Invitrogen, Paisley, UK
Ethanol	Merck, Darmstadt, Germany
Ethidium bromide	Sigma-Aldrich. St.Louis, MO, USA
Fetal bovine serum (FBS)	HyClone, Cramlington, UK
GENETIC SYSTEM™ HIV-1 Ag EIA kit	Bio-Rad Laboratories, Redmond, WA, USA
Glacial acetic acid	BDH Laboratory Supplies, UK
Glycerol	Sigma-Aldrich. St.Louis, MO, USA
High Pure PCR Template Preparation Kit	Roach, Mannheim, Germany
His-bind column chromatography	Novagen, San Diego, CA, USA

Chemical name	Source
Hybond-P polyvinylidene fluoride (PVDF) membrane	Amersham Bioscience, Piscataway, NJ, USA
Imidazole	Sigma-Aldrich. St.Louis, MO, USA
isopropyl β-*D*-thiogalactopyranoside (IPTG) , dioxan-free	Fermentas, Burlington, ON, Canada
Kanamycin	Sigma-Aldrich. St.Louis, MO, USA
LB Broth Agar	Bio Basic inc., Ontario, Canada
L-glutamine	Gibco, Grand Island, NY, USA
Lipofectamine	Invitrogen, Carlsbad, CA, USA
Methanol	Merck, Darmstadt, Germany
Micro-BCA protein assay	Pierce, Rockford, lL, USA
Millipore Millex-HA filter unit, 0.45µm	Millipore, Cork, Ireland
NaCl	Sigma-Aldrich. St.Louis, MO, USA
NaOH	Sigma-Aldrich. St.Louis, MO, USA
Nucleofector™ transfection reagent V	Lonza, Basel, Switzerland
Paraformaldehyde	Sigma-Aldrich. St.Louis, MO, USA
Plus Reagent	Invitrogen, Carlsbad, CA, USA
polybrene	Sigma-Aldrich. St.Louis, MO, USA
PureLink Quick Plasmid Miniprep Kit	Invitrogen, Carlsbad, CA, USA
QIAGEN Miniprep Kit	Qiagen, Hilden, Geramany

Chemical name	Source
Sensor chip SA	Biacore AB, Uppsala, Sweden
TEMED	Biorad, Hercules, CA, USA
Tetracyclin	Sigma-Aldrich. St.Louis, MO, USA
Triton X-100	Sigma-Aldrich. St.Louis, MO, USA
Trypan Blue 0.2%	Sigma-Aldrich. St.Louis, MO, USA
Tryptone water	Merck, Darmstadt, Germany
Tween 20	Fluka, Buchs, Switzerland
Yeast extract	Bio Basic inc., Ontario, Canada

2. Instruments

Instruments	Source
37 °C CO_2 incubator EG 115 IR	Jouan GmbH, Unterhaching, Germany
37 °C incubator	JP Selecta, Barcelona, Spain
BioRad Chemidoc XRS Gel Documentation System	BioRad, Hercules, CA, USA
BECKMAN L-60 ultracentrifuge	Beckman Coulter, Fullerton, CA, USA
Carl Zeiss MicroImaging LSM 700 confocal laser scanning microscope	Carl Zeiss MicroImaging GmbH, Germany
Electrophoretic power supply 3000Xi	BioRad, Hercules, CA, USA
Flow cytometer (BD FACSCalibur™)	BD Biosciences, San Diego, CA, USA
Fluorescence microscopy, OLYMPUS AX70	Olympus, Tokyo, Japan
Shaking incubator (JSSI-100C)	JS Research Inc., Gongju-city, Koria
Inverted fluorescence microscope	Nikon eclipse TE2000-S, Japan
Inverted microscope	Olympus, Japan
Laminar Flow biological safety cabinet	NUAIRE, Plymouth, MN, USA
Microcentrifuge	Eppendorf AG, Hamburg, Germany
Microplate	NUNC, Roskilde, Denmark
MiniVE vertical electrophoresis system	Amersham Pharmacia Biotech, Buckinghamshire, UK

Instruments	Source
MJ Mini™ Thermal Cycler and MiniOpticon™ Real-Time PCR System	BioRad, Hercules, CA, USA
MRX-150 Refrigerated microcentrifuge	Tomy Tech USA Inc., CA, USA
MTP-120 ELISA plate reader	Corona Electric, Japan
NanoDrop 2000	ThermoScientific, Rockford, IL, USA
Nucleofector™	Amaxa, Koeln, Germany
Typhoon Trio phosphorImager	GE Healthcare Biosciences, Piscataway, NJ
Ultrasonic Processor UP100H	Hielscher, Teltow, Germany
UV spectrophotometer	Shimadzu Scientific Instruments Inc, Kyoto, Japan
UV-2450/2550 spectrophotometer	Shimadzu, Columbia, MD, USA
Vortex-Genie K-550-GE	Scientific Industries Inc, Bohemia, NY, USA

APPENDIX B

LIST OF CELL LINES AND MICROORGANISMS

1. Cell lines

Name	Type of cell lines
HeLa	Cervical carcinoma cell
Sup T1	Human T cell lymphocytic cell line
293T	Human embryonic kidney cell

2. Microorganisms

2.1 *Escherichi coli* XL-1 Blue MRF'

Genotype: Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)*173 endA1 supE44 thi-1 recA1* gyrA96 *rel*A1 *lac [F'* proAB lacI^{q}Z*Δ*M15 *Tn*10 *(Tet^{r})]*

2.2 *Escherichi coli* BL21 (DE3)

Genotype: F^{-} *omp*T *gal dcm lon hsd*S_B (r_B^- m_B^-) λ(DE3 [*lacI lac*UV5-T7 *gene 1 ind1 sam7 nin5*])

APPENDIX C

LIST OF ANTIBODIES AND CONJUGATED ANTIBODIES

Antibodies name	Source
Anti-CD4 mAb	Kindly provided by Prof. Dr. Watchara Kasinrerk
Anti-His tag antibody	GenScript, Piscataway, NJ, USA
Horseradish peroxidase (HRP) - labeled goat anti-mouse immunoglobulins	Sigma, St Louis, MO,USA
Polyclonal Rabbit Anti-mouse Immunoglobulins/RPE, Rabbit $F(ab')_2$	DAKO, Denmark

APPENDIX D

LIST OF ENZYMES

Enzymes	Sources
Accuprime™ Pfx DNA polymerase	Invitrogen, San Diego, CA
DyNAmo^{TM} probe qPCR	Finnzymes, Espoo, Finland
Nhe I	NEB, Pickering, Ontario, USA
Not I	NEB, Pickering, Ontario, USA
5 PRIME MasterMix (2.5X)	5 Prime, Gaithersburg, MD, USA
Sma I	Fermentas, Glen. Burnie, MD, USA
T4 DNA ligase enzyme	NEB, Pickering, Ontario, USA
Xcm I	NEB, Pickering, Ontario, USA

APPENDIX E

REAGENT PREPARATIONS

1. Reagents for gel electrophoresis

1.1 10× Tris-acetate/EDTA electrophoresis buffer (TAE)

Tris-base	48.40	gm
Glacial acetic acid	11.42	ml
0.5 M EDTA, pH 8.0	20	ml

Dissolved all ingredients in deionized distilled water and filled up to 1,000 ml.

Sterilized by autoclave and kept at room temperature.

1.2 1 or 2 % Agarose gel

Agarose	1 or 2	gm
1× TAE	100	ml

Melted by microwave oven until the agarose was completely dissolved.

1.3 Ethidium bromide working solution (10 mg/ml)

Ethidium bromide	1.0	gm
Distilled water	100	ml

Dissolved and kept in dark bottle at 4 °C.

1.4 6X gel loading buffer

Bromphenol blue	0.25	%
Glycerol	30	%

Mixed thoroughly and stored at -20 °C.

2. Reagents for SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting

2.1 1.5 M Tris-HCl, pH 8.8

Tris-base	18.15	gm

Dissolved in 75 ml deionized distilled water.

Adjusted pH to 8.8 with concentrated HCL.

Adjusted the volume to 100 ml with deionized distilled wate

stored at 4 °C.

2.2 0.5 M Tris-HCl, pH 6.8

Tris-base	6.0	gm

Dissolved in 75 ml deionized distilled water.

Adjusted pH to 6.8 with concentrated HCl.

Adjusted the volume to 100 ml with deionized distilled water

and stored at 4 °C.

2.3 Running buffer

Tris-base	1.51	gm
Glycine	7.20	gm
Sodium dodesyl sulfate	0.5	gm

Dissolved in 500 ml deionized distilled water and kept at 4 °C.

2.4 Blotting buffer

Tris-base	3.03	gm
Glycine	14.41	gm
SDS	0.5	gm

Added deionized distilled water to 700 ml and mixed well.

Added 200 ml of methanol

Adjusted the volume to 1,000 ml with deionized distilled water and kept at 4 °C.

2.5 Copolymerization of 4% stacking gel (5 ml)

Stock acrylamide 30%	0.83	ml
0.5 M Tris-HCl pH 6.8	0.63	ml
10% SDS	0.05	ml
DW	3.40	ml
10% Ammonium persulfate	0.05	ml
TEMED	0.01	ml

2.6 Copolymerization of 12% stacking gel (10 ml)

Stock acrylamide 30%	4.00	ml
Gel buffer pH 8.8	2.50	ml
10% SDS	0.10	ml
DW	3.30	ml
10% Ammonium persulfate	0.10	ml
TEMED	0.01	ml

3. Reagents for Electrophoretic mobility shift assay (EMSA)

3.1 Running buffer (10X TB buffer, pH 8.2)

Tris-base	108	gm
Boric acid	55	gm

Dissolved in 750 ml deionized distilled water.

Adjusted pH to 8.2 with concentrated HCL.

Adjusted the volume to 1000 ml with deionized distilled water

stored at 4 °C.

3.2 Copolymerization of 5% Native gel (60 ml)

Stock 29:1 acrylamide/Bis (w/w)	10.0	ml
10 X TBE buffer	3.0	ml
DW	47.0	ml
10% Ammonium persulfate	0.25	ml
TEMED	0.034	ml

4. Reagents for surface plasmon resonance (SPR)

4.1 Washing solution (50 mM NaOH/ 1M NaCl)

5 M NaOH	1.0	ml
5 M NaCl	20	ml

Adjusted the volume to 100 ml with deionized distilled water

Filtered with 0.2 μm Millipore filter and stored at 4 °C.

4.2 Zinc buffer (200 ml)

1.5 M Tris-HCl, pH 7.5	1.33	ml
0.5 M KCl	36.0	ml
1 M $MgCl_2$	0.2	ml
100 mM $ZnSO_4$	180	μl
100 mM dithiothreitol (DTT)	10	ml
100 mM phenylmethylsulfonylfluoride (PMSF)]	1	ml

Adjusted the volume to 200 ml with distilled water

Filtered with 0.2 μm Millipore filter stored at 4 °C.

3. Medium for bacterial culture

3.1 50% glucose

D-glucose	5	gm

Added distilled water to 10 ml and boiled in boiling water.

Filtered through 0.2 μm Millipore filter and stored at 4 °C.

3.2 LB broth

Yeast extract	5.0	gm
Tryptone	10.0	gm
NaCl	10.0	gm

Dissolved all ingredients in 1,000 ml distilled water

Sterilized by autoclave, and kept at 4 °C.

3.3 LB agar

LB agar	15	gm

Dissolved all ingredients in 1,000 ml distilled water.

Sterilized by autoclave, poured on Petri dish (plate) and stored at 4 °C.

3.4 Terrific broth

Tryptone	12	gm
Yeast extract	24	gm
Glycerol	4	ml

Adjusted to in 900 ml with distilled water.

Sterilized by autoclave and allowed to cool to room temperature

Adjust volume to 1,000 ml with 100 ml of a filter sterilized solution of

0.17M KH_2PO_4 and 0.72M K_2HPO_4

4. Reagents for fluorescence microscopy and flow cytometry analysis

4.1 4% Paraformaldehyde in PBS

Paraformaldehyde	4	gm
PBS pH 7.2	100	ml

Heat at 56˚C until dissolved

Filtered with 0.2 μm Millipore filter, stored at 4˚C.

4.2 1% BSA-PBS-NaN_3

BSA	1	gm
NaN_3	0.09	gm

Dissolved in PBS 100 ml

4.3 0.2% Triton X-100

Triton X-100	0.2	ml

Dissolved in PBS 100 ml

5. Reagents for cell culture

5.1 RPMI 1640 medium

RPMI powder	1	pack
$NaHCO_3$	2	gm
ddH_20	800	ml
Penicillin (10,000 units/ml)/Streptomycin (10,000 μg/ml)	1	ml

Stirred until dissolved and adjusted pH with acetic acid.

Dissolved in ddH_20 and adjust volume to 1,000 ml.

Filtered through 0.2 µm Millipore membrane filter.

Mixed and stored at 4 ºC.

5.2 Complete RPMI culture medium

RPMI 1640 medium	90	ml
Fetal bovine serum (FBS)	10	ml

Checked sterility before used.

5.3 Freezing medium (10%DMSO in 90%FCS)

Fetal bovine serum (FBS)	9	ml
DMSO	1	ml

Fresh preparation before use.

5.4 Trypan blue (0.2%)

Trypan blue powder	0.2	gm
PBS pH 7.2	100	ml

Filtered by Whatman filter paper No. 1 and

Stored at room temperature.

APPENDIX F

PRESENTATIONS AND PUBLICATIONS

List of presentation

1. Designed Zinc Finger Protein Interacting with the HIV-1 Integrase Recognition Sequence at 2-LTR-Circle Junctions. 3rd Annual meeting of the Faculty of Associated Medical Sciences Chiang Mai, Thailand. 11 June 2009 (Excellent oral presentation award).

2. Designed Zinc Finger Protein Interacting with the HIV-1 Integrase Recognition Sequence at 2-LTR-Circle Junctions. RGJ seminar series LXXIV "From basic biomedical research to sustainable development". Chiang Mai, Thailand. 16 September 2010 (Poster presentation).

3. Designed Zinc Finger Protein Interacting with the HIV-1 Integrase Recognition Sequence at 2-LTR-Circle Junctions. The Fogarty International Clinical Research Scholars and Fellow and Doris Duke International Fellows Alumni Symposium. Bolger Center, Potomac, MD, USA. September 23 - 26, 2010 (Poster presentation)

4. The Innovative Strategy for HIV-1 Gene Therapy by Zinc Finger Protein. The 28th Annual Meeting of Allergy, Asthma, and Immunology Association of Thailand. Plaza Athenee, Bangkok, April 3-5, 2012 (Winner of MSD Investigator Awards)

List of publications

1. **Sakkhachornphop, S**., Jiranusornkul, S., Kodchakorn, K., Nangola, S**.**, Sirisanthana, T. and Tayapiwatana, C. (2009) Designed zinc finger protein interacting with the HIV-1 integrase recognition sequence at 2-LTR-circle junctions. Protein Sci 18, 2219-30.

Impact Factor 2.937

2. **Sakkhachornphop, S**., Barbas III, C., Keawvichit, R., Wongworapat, K. and Tayapiwatana, C. (2012) Zinc Finger Protein Designed to Target 2-LTR Junctions Interferes with HIV Integration. Human Gene Therapy, (accepted)

Impact Factor 4.829

CURRICULUM VITAE

Name	Mr Supachai Sakkhachornphop
Date of birth	July 12th, 1975
Education	
1993-1996	Bachelor of Science (Medical Technology), Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand
2003-2006	Master of Science (Health Sciences), The Graduate School, Chiang Mai University, Chiang Mai, Thailand
Working experiences	
1997 – 1999	Medical Technologist, Central Chiang Mai Memorial Hospital, Chiang Mai, Thailand
1999 – present	Medical Technologist and Research Assistance, The Research Institute for Health Sciences, Chiang Mai University, Chiang Mai, Thailand

Practical training and research fellowships

2004	Training in HIV subtyping at U.S. Military HIV Research Program (USMHRP), Henry M. Jackson Foundation, Rockville, MD, USA
2004-2005	Research Fellow of the Fogarty/Ellison Fellowship Program in global health and clinical research.
2007-2009	Research Fellow of the Fogarty International Clinical Research.
2009	Training in zinc finger protein and lentiviral gene transfer at the Scripps Research Institute, San Diego, CA, USA.

List of publications and presentations

1. **Sakkhachornphop S**, Pichayangkul S, and Hirunpetcharat C. 2000.Production and purification of the 19- kDa carboxyl-terminal fragment of *Plasmodium yoelii* merozoite surface protein-1 ($MSP1_{19}$) as a flag fusion protein expressed in *Saccharomyces cerevisiae.Bull Chiang Mai Assoc Med Sci* ;33 : 161-71.
2. Hirunpetcharat C, **Sakkhachornphop S**, Pichyangkul S, Krieg AM, and Good MF. Protective immunity induced in mice by parenteral immunization with yeast-expressed 19 kDa carboxyl terminal fragment of *Plasmodium yoelii* merozoite surface protein-1 ($MSP1_{19}$) using montanide 51, montanide 720 and CpG oligodeoxynucleotides as adjuvants. Presented at the 18th Annual Health Sciences Meeting. June 8, 2000. Reserch Institute for Health Sciences. Chiang Mai University.
3. Chearwae W, **Sakkhachornphop S**, Chumpookhod A, Chumpookhod S, Palanan P, Tejafong K, Kitisri J, and Chanbancherd P. Application of dried blood spots in molecular epidemiological study of HIV-1. Presented at the 19th Annual Health Sciences Meeting. August 24, 2001. Research Institute for Health Sciences. Chiang Mai University.
4. Hirunpetcharat C, Wipasa J, **Sakkhachornphop S**, Nitkumhan T, Zheng YZ, Pichyangkul S, Krieg AM, Walsh DS, Heppner DG, and Good MF. CpG oligonucleotide enhances immunity blood-stage malaria infection in mice parenterally immunized with a yeast-expressed 19 kDa carboxyl-terminal fragment of *Plasmodium yoelii* merozoite surface protein-1 ($MSP1_{19}$) formulated in oil-based Montanides. Vaccine 2003; 21: 2923-32.

5. Tovanabutra S, Beyrer C, **Sakkhachornphop S**, *et al*. The Changing Molecular Epidemiology of HIV-1 among Northern Thai Drug Users, 1999 to 2002. AIDS Res Hum Retroviruses 2004; 20 :Number 5 : Issuue date May 2004

6. **Sakkhachornphop S**, Tovanabutra S, Kijak G, *et al*. Development and Application of the Multi-region Subtype Specific PCR (MSSP) Assay for HIV-1 Subtypes B, C, CRF01_AE, Their Recombinant Forms, and Dual Infections. Oral presentation at the 13th HIV Dynamics and Evolution. April 5-8, 2006.Marine Biology Labs. Woods Hole. MA. USA.

7. Quan V.M., Celentano D.D., Rungruengthanakit K, Pasawad W, Vongchak T, **Sakkhachornphop S**, *et al*. Decline in CD4+ T lymphocyte count among substance abuse patients within one year after HIV-1 infection. Poster presentation at the XVI International AIDS Conference. August 13-18, 2006. Toronto. Canada.

8. Tovanabutra S, Kijak G, Beyrer C, Gammon-Richardson C, **Sakkhachornphop S**, *et al*. The link between multiple HIV-1 exposure and genetic complexity of strains is reinforced by identification of a second circulating recombinant form among injecting drug users in northern Thailand. Poster presentation at the XVI International AIDS Conference. August 13-18, 2006. Toronto. Canada.

9. **Sakkhachornphop S**, Tovanabutra S, Kijak G, *et al*. Screening for Circulating Recombinant Forms of HIV-1 among Northern Thai Drug Users with a Multi-region Subtype Specific PCR (MSSP) Assay. Poster presentation at the 5 th Joint Seminar on Biomedical Sciences. October 26-27, 2006. Kunming. China.

10. Tovanabutra S, Kijak G, Beyrer C, Gammon-Richardson C, **Sakkhachornphop S**, *et al*. Identification of CRF34_01B, a second circulating recombinant form unrelated to and more complex than CRF15_01B, among injecting drug users in northern Thailand. AIDS Res Hum Retroviruses. 2007 Jun; 23(6):829-33.

11. **Sakkhachornphop S**, Tovanabutra S, Kijak G, *et al*. Development and Application of the Multi-region Subtype Specific PCR (MSSP) Assay for HIV-1 Subtypes B, C, CRF01_AE, Their Recombinant Forms, and Dual Infections. (in preparation)

12. Utaipat U, Ketkarn J, **Sakkhachornphop S**, *et al*. Duration of infection and low CD4:CD8 ratio correlate with CXCR4 utilization among HIV-1 subtype CRF01-AE. Poster presentation at the 4th IAS Conference on HIV Pathogenesis, Treatment, and Prevention incorporating the 19 th ASHM Conference. July 22-25, 2007. Sydney. Australia.

13. **Sakkhachornphop S**, Tovanabutra S, Kijak G, *et al*. Development and Application of the Multi-region Subtype Specific PCR (MSSP) Assay for HIV-1 Subtypes B, C, CRF01_AE, Their Recombinant Forms, and Dual Infections. Oral presentation at the Fogarty International Clinical Research Scholars Program's Scientific Session. March 9 - 11, 2008. Marriott Bethesda and National Institutes of Health, Bethesda, MD, USA.

14. **Sakkhachornphop S,** Jiranusornkul, Kodchakorn K, Nangola S, Sirisanthana T, and Tayapiwatana C**.** Designed Zinc Finger Protein Interacting with the HIV-1 Integrase Recognition Sequence at 2-LTR-Circle Junctions. Protein Sci. 2009 Nov; 18(11):2219-30.

15. **Sakkhachornphop S,** Jiranusornkul, Kodchakorn K, Nangola S, Sirisanthana T, and Tayapiwatana C**.** Designed Zinc Finger Protein Interacting with the HIV-1 Integrase Recognition Sequence at 2-LTR-Circle Junctions. Poster presentation at the Fogarty International Clinical Research Scholars and Fellow and Doris Duke International Fellows Alumni Symposium. September 23 - 26, 2010. Bolger Center, Potomac, MD, USA.

16. Wipasa J, Okell L, **Sakkhachornphop** S, Suphavilai C, Chawansuntati K, Liewsaree W, Hafalla JC, Riley EM. Short-lived IFN-γ effector responses, but long-lived IL-10 memory responses, to malaria in an area of low malaria endemicity. PLoS Pathog. 2011 Feb 10; 7(2):e1001281.