APPENDIX A

List of Chemicals, Reagents and Materials Used in This Study

Chemicals, reagents and materials	Source
1.5-mL microcentrifuge tube	Labcon, USA
10-, 20-, 200- and 1,000-uL filter tip	Labconnection, Thailand
100x Penicillin-Streptomycin	GIBCO, USA
10-mL disposable syringe	NIPRO, Japan
10-mL sodium heparin tube	BD, USA
15- and 50 centrifuge tube	Labcon, USA
21G needle	NIPRO, Japan
25 or 75-cm^2 T-flask	Nunc
Absolute ethanol	RCl Labscan, Thailand
Agarose	CONDA, Spain
Anti-Digoxigenin-AP, Fab fragments	Roche, Switzerland
Bovine Serum Albumin (BSA) Fraction V	PAA, Austria
Bromphenol blue	Ajax Finechem, Australia
CDP-star™ Detection Reagent	GE Healthcare, UK
Concentrated (37%) HCl	BDH, UK
Concentrated (85%) H_3PO_4	Merck, Germany
Disodium hydrogen phosphate dihydrate	APS, Australia
DNA Molecular Weight Marker III (0.12-21.0 kbp)	Roche, Switzerland
DNA Molecular Weight Marker III VII (0.081-8.57 kbp)	Roche, Switzerland
DNA Ultra Polymerase	PCRBIO, UK
-5X buffer (Supplied with DNA Ultra Polymerase)	
dNTP mixture	Vivantis, USA
Dulbecco's modified Eagle's medium	GIBCO, USA
E.Z.N.A.® Gel extraction Kit	OMEGA bio-tek, USA

Chemicals, reagents and materials	**Source**
EDTA	Vivantis, USA
Ethidium bromide	Amresco, USA
Fetal bovine serum	GIBCO, USA
	GE Healthcare, UK
Glacial acetic acid	USB, USA
*Hinf*I	ThermoFisher, USA
- 10x Tango buffer (Supplied with *Hinf*I)	
Immobilon-Ny+ Membrane	Merck, Germany
Magnesium Chloride	BDH, UK
Maleic acid	Bio Basic Inc., Canada
Max *Taq* DNA Polymerase	Vivantis, USA
-10X buffer (Supplied with Max *Taq* DNA Polymerase)	
Medical X-ray Green/ MXG Film	Carestream Health, USA
Membrane filter pore size 0.45 µm	Millipore, Merck
Non-fat powdered milk	Bio Basic Inc., Canada
Nuclease free water	OMEGA bio-tek, USA
Nylon membrane	Millipore, USA
Oligonucleotides and probe	Bio Basic Inc., Canada
	IDT, USA
PCR tube	Proline
Phosphate buffer saline (PBS)	GIBCO, USA
Proteinase K	Vivantis, USA
RedSafe™ DNA staining	iNtRON, Korea
*Rsa*I	ThermoFisher, USA
-10x Tango buffer (Supplied with Taq DNA Polymerase)	
Sodium chloride	Merck, Germany
Sodium dodecyl sulfate	Riedel-de Haën, Sigma
Sodium hydroxide	Merck, Germany
Sucrose	Merck, Germany
Taq DNA Polymerase	Vivantis, USA
-10X buffer (Supplied with Taq DNA Polymerase)	

Chemicals, reagents and materials	Source
Tris-base	Vivantis, USA
Trisodium citrate	Bio Basic Inc., Canada
Trypsin-EDTA	GIBCO, USA
Tween-20	BDH, UK
VC 100 bp plus DNA ladder	Vivantis, USA
Xlarge DNA ladder	GeneDirex, China
XPRESS SYBR®GreenER™ qPCR Super Mix	ThermoFisher, USA

APPEXDIX B

List of Instrument Used in This Study

Instrument	Source
ABI 7500	Applied Biosystems, USA
Automatic pipette	GILSON, USA
	Eppendorf, Germany
Centrifuge SC-15AR	TOMY SEIKO, Japan
Cubee Mini-Centrifuge	GeneReach, Taiwan
Film cassette	Fisher Scientific, USA
Freezer (-20˚C)	SAMSUNG, Korea
Freezer (-80˚C)	Forma Scientific, USA
Gel Documentation System	Synergy G, Germany
GeneQuant™ pro RNA/DNA Spectrophotometer	GE Healthcare, UK
HICLAVE HV-85	HIRAYAMA, Japan
Horizontal gel electrophoresis system	Mupid®-eXu, Japan
Hot air oven	Hareus
Hybaid Limited Equipment Class 1	UK
Laminar Flow Biosafety Cabinet	Labguard NU-440-400E
Light microscope	Olympia, Tokyo
Magnetic stirrer	Lab Tech
Masterclycler	Eppendorf, Germany
Mastercycle gradient thermal cycler	BIO-RAD, UK
Microwave	LG, Korea
MyGene™ Series Peltler Thermal Cycler Model MG25+	LongGene, China
pH meter	Proline
Refrigerated centrifuge H-103N SERIES	KOKUSAN, Japan
Refrigerator	HITACHI, Japan

Instrument	Source
Rocker platform	Bellco Glass, US
Typhoon phosphorimager	Typhoon, USA
VacuGene XL Vacuum Blotting system	Amersham bioscience
Vortex	Scientific Industry, USA
Water bath	Science/Electronic, USA

APPEXDIX C

Preparation of Reagents and Buffers

Stock reagents

1. Stock 1 M Tris-HCl, 100 mL

Tris-base	12.11	g
Sterile distilled water	80	mL

Adjust pH to 7.2, 7.6 and 8.0 by adding HCl, then adjust volume to 100 ml and filtrate any nonsoluble powder by filtration with membrane filter pore size 0.45 μm, collecte in dark container.

2. Stock 5 M $MgCl_2$, 100 mL

$MgCl_2$	47.60	g
Sterile distilled water	80	mL

Adjust volume to 100 ml and filtrate any nonsoluble powder by filtration with membrane filter pore size 0.45 μm.

3. Stock 5 M NaCl, 100 mL

NaCl	29.22	g
Sterile distilled water	80	mL

Adjust volume to 100 ml and filtrate any nonsoluble powder by filtration with membrane filter pore size 0.45 μm.

4. Stock 0.5 M EDTA, pH 8.0, 100 mL

EDTA	18.612	g
Sterile distilled water	80	mL

Adjust pH to 8.0 with NaOH, then adjust volume to 100 ml and filtrate any nonsoluble powder by filtration with membrane filter pore size 0.45 μm.

5. Stock 50x Tris-acetate-EDTA (TAE) buffer, 1 L

Tris base	242	g
Glacial acetic acid	57.1	mL
0.5 M EDTA, pH 8.0	100	mL

Adjust volume to 1 L and filtrate any nonsoluble powder by filtration with membrane filter pore size 0.45 μm.

6. Stock 20% sodium dodecyl sulfate (SDS), 1 L

SDS power	200	g
Distilled water	800	mL

Heat at 68oC to assist dissolution, then adjuste volume to 1 L

7. Stock 6x gel loading buffer, 10 mL

Bromophenol blue	25	mg
Sucrose	4	g

Adjust volume to 10 mL and keep at 4^{o}C

8. Stock 10 mg/mL ethidium bromide, 10 mL

Ethidium bromide	0.1	g
Distilled water to	10	mL

Keep at room temperature in a brown bottle

9. Stock 20 mg/mL Proteinase K, 1 mL

Proteinase K power	20	mg
Sterile distilled water	1	mL

Aliquot and keep at -20^{o}C

10. Stock 2.5 M $Na_2HPO_4 \cdot 2H_2O$, 100 mL

$Na_2HPO_4 \cdot 2H_2O$	44.5	mg

Adjust volume to 100 mL with sterile distilled water and filtrate any nonsoluble powder by filtration with membrane filter pore size 0.45 μm.

11. Stock 20x SSC solution, 1 L

NaCl	175	g
Trisodium citrate	88.2	g

Adjust volume to 1 L with sterile distilled water and filtrate any nonsoluble powder by filtration with membrane filter pore size 0.45 μm.

12. Stock 2x Maleic acid solution

Maleic acid	23.22	g
NaCl	17.55	g
NaOH	14.0	g

Adjust volume to 1 L with sterile distilled water and filtrate any nonsoluble powder by filtration with membrane filter pore size 0.45 μm.

Human cancer cell culture

1. Incomplete DMEM, 1 L

DMEM powder	1	package
Deionize distilled water	800	ml

Adjust pH to 7.2-7.4, then adjust volume to 1,000 ml and sterile by suction filter (membrane pore size 0.2 μM).

2. Completed DMEM medium, 500 mL

Incomplete DMEM medium	449	ml
Fetal bovine serum	50	ml
100x Pen-Strep	5	ml

Store at 4˚C

3. 1x Phosphate buffer saline (PBS), pH 7.4, 1 L

PBS power	1	package

Adjust volume to 1 L with distilled water, autoclave, and keep at 4^{o}C

Genomic DNA extraction

1. Red Cell Lysis Buffer (RCLB), 1 L

Stock 1 M Tris-HCl, pH 7.6	10	mL
Stock 1 M $MgCl_2$	5	mL
Stock 5 M NaCl	2	mL

Adjust volume to 1 L with sterile distilled water and filtrate with membrane filter pore size 0.45 μm.

2. Nuclei Lysis Buffer (NLB), 1 L

Stock 1 M Tris-HCl, pH 8.0	10	mL
Stock 5 M NaCl	80	mL
Stock 0.5 M EDTA, pH 8.0	4	mL

Adjuste volume to 1 L with sterile distilled water and filtrate with membrane filter pore size 0.45 µm.

3. Proteinase K solution, 0.5 mL

Stock 20 mg/mL Proteinase K	50	µL
Stock 20% SDS	25	uL
Stock 0.5 M EDTA, pH 8.0	2	uL

Adjust volume to 0.5 mL

4. 70% Ethanol, 1 L

Absolute ethanol	700	mL
Sterile distilled water	300	mL

5. Tris-EDTA (TE) buffer, pH 8.0, 50 mL

Stock 1 M Tris-HCl, pH 8.0	0.5	mL
Stock 0.5 M EDTA, pH 8.0	0.1	mL

Adjuste volume to 50 mLwith sterile distilled water and filtrate with membrane filter pore size 0.45 µm.

Gel electrophoresis

1. Working 1x TAE buffer, 1 L

Stock 50x TAE buffer	20	mL

Adjuste volume to 1 L with distilled water

2. 0.8 % TAE agarose gel

Agarose power	0.8	g
Working 1x TAE buffer, pH 8.0	100	mL

Melt the suspension with microwave

3. Working 0.5 ug/mL ethidium bromide, 200 mL

Stock 10 mg/mL ehtidium bromide	10	µL
Distilled water	200	mL

Southern blot analysis

1. Alkaline transfer buffer, 1 L

NaOH	16	g
NaCl	58.4	g

Adjust volume to 1 L with sterile distilled water and filtrate with membrane filter pore size 0.45 µm.

2. Neutralizing solution, 50 mL

Stock 1 M Tris-HCl, pH 7.2	25	mL
Stock 5 M NaCl	10	mL

Adjust volume to 50 mL with sterile distilled water and filtrat with membrane filter pore size 0.45 µm.

3. Pre-hybridizing solution (Church Buffer), 1 L

Stock 2.5 M $Na_2HPO_4 \cdot 2H_2O$	200	mL
Stock 0.5 M EDTA, pH 8.0	2	mL
Concentrated (85%) H_3PO_4	4	mL
Stock 20% SDS	350	mL
1% w/v BSA	200	mL

Adjust volume to 1 L with sterile distilled water and keep at -20ºC

4. Stringent wash buffer I, 1 L

Stock 20x SSC solution	100	mL
Stock 20% SDS	5	mL

Adjust volume to 1 L with sterile distilled water

5. Stringent wash buffer II, 1 L

Stock 20x SSC solution	10	mL
Stock 20% SDS	5	mL

Adjust volume to 1 L with sterile distilled water

6. Stringent wash buffer II, 1 L

Stock 20x SSC solution	10	mL
Stock 20% SDS	5	mL

Adjust volume to 1 L with sterile distilled water

7. 1x Maleic acid solution, 1 L

Stock 2x Maleic acid solution	500	mL
Sterile distilled water	500	mL

8. Washing solution, 1 L

Stock 2x Maleic acid solution	500	mL
Tween 20	3	mL

Adjust volume to 1 L with sterile distilled water

9. Blocking solution, 50 mL

Non-fat powdered milk	0.5	g

Adjust volume to 1 L with 1x Maleic acid solution

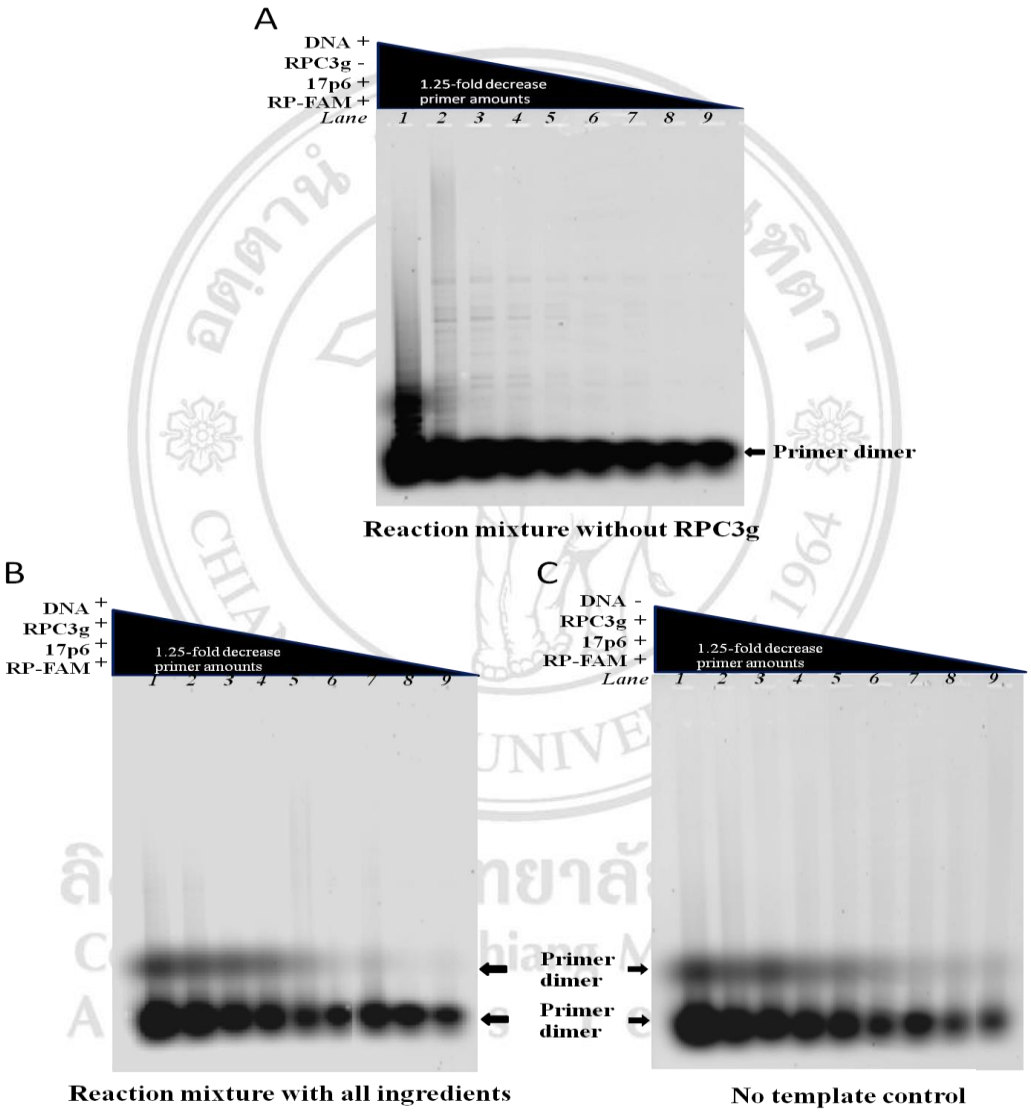
10. 1x Detection solution, 1 L

Tris base	24.22	g
NaCl	11.68	g
Sterile distilled water	900	mL

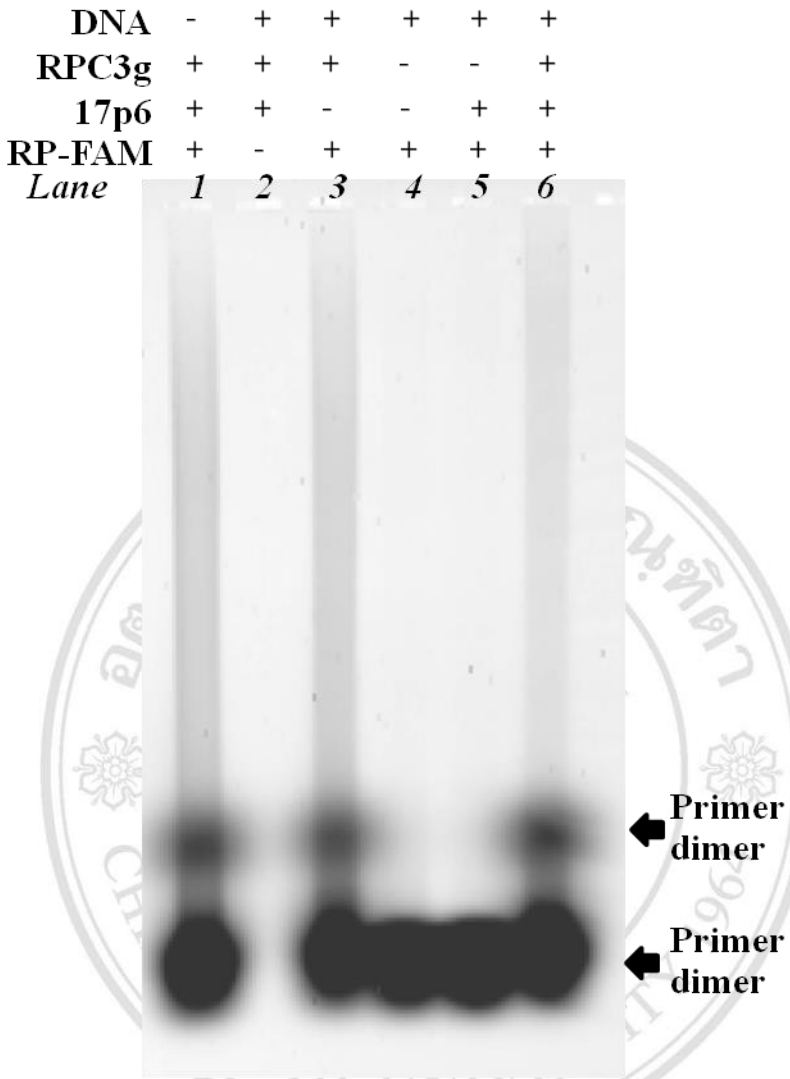
Adjust pH to 9.5 with concentrated (37%) HCl, and adjust volume to 1 L with sterile distilled water

APPEXDIX D

Supplementary Data

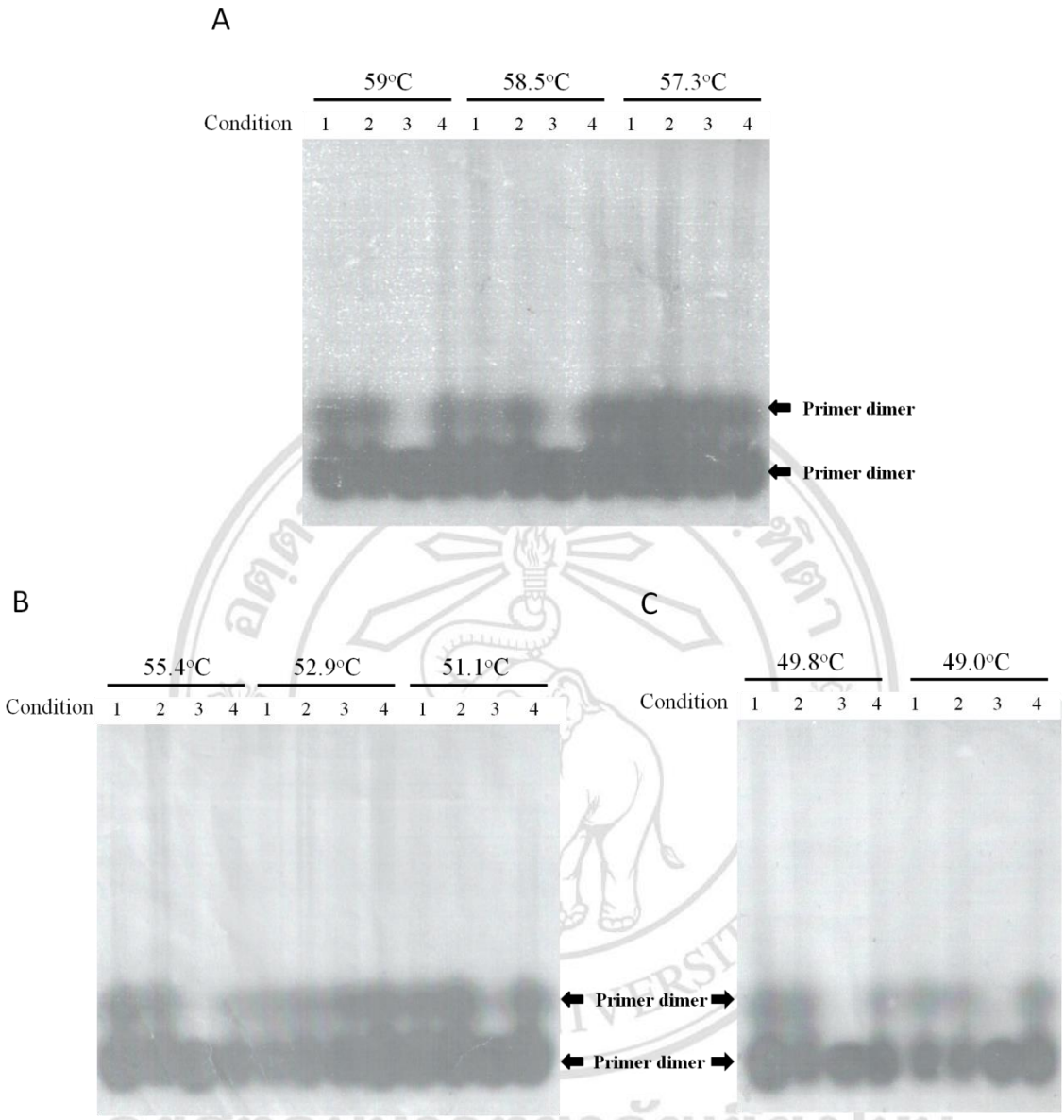


Supplementary data S1 The 1.25-fold decrease amounts of RP-FAM and 17p6 The products were resolved by 0.8% TAE agarose gel electrophoresis and directly detected their fluorescence signal by phosphoimager. + and – represent the reagent added and depleted in the reaction mixture, respectively



Supplementary data S2 Separation of 3’ telomeric end modification and Chr17p telomere amplification steps

The RPc3g was allowed to modify 3’ telomere DNA before adding the 5’ FAM-labeled RP and 17p6 into reaction mixture. The products were resolved by 0.8% TAE agarose gel electrophoresis and directly detected their fluorescence signal by phosphoimager. + and – represent the reaction mixture with and without the indicated ingredient, respectively



Supplementary data S3 The gradient PCR for RPC3g suitable temperature in modifying 3’ telomeric DNA

The temperature was ranged from 49 to 59ºC before 59ºC was used to amplify Chr17p telomeres. The products were resolved by 0.8% TAE agarose gel electrophoresis and directly detected their fluorescence signal by phosphoimager. 1, 2, 3 and 4 represent no template control, reaction mixture without 17p6, reaction mixture without RPC3g and reaction mixture with all components, respectively.

CURRICULUM VITAE

Name		Mr. Surasit Bupachat
Date of birth		August 3, 1982
Education	1995-1997	Certificate of secondary school at Sala Wittaya School, Lampang, Thailand
	1998-2000	Certificate of high school at Lampang Kunlayanee School, Lampang, Thailand
	2001-2004	B. Sc. (Medical Technology), Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand