

## **APPENDICES**

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

Copyright© by Chiang Mai University  
All rights reserved

## Appendix A

### List of Chemicals

#### Chemicals

#### Sources

Absolute ethanol

Merck, Germany

Agarose

Research Organics Inc., USA

Boric acid

Sigma-Aldrich Inc., Germany

Bromophenol Blue

Amresco, USA

BigDye Terminator Sequencing kit

Applied Biosystems, USA

Chelex-100 (iminodiacetic acid)

Sigma-Aldrich Inc., Germany

DMSO

Sigma-Aldrich Inc., Germany

1-kb DNA ladder

Fermentas, Canada

$\lambda$  Hind III DNA ladder

Fermentas, Canada

DreamTaq DNA polymerase

Fermentas, Canada

ΦX 174 DNA ladder	Fermentas, Canada
EDTA	Bio Basic Inc., Canada
Ethanol	Sigma-Aldrich Inc., Germany
Ethidium bromide	Bio Basic Inc., Canada
Ficoll PM 400	Amersham Biosciences, Sweden
Glycerol	Sigma-Aldrich Inc., Germany
i-Taq DNA polymerase	Intron Biotechnology Inc., Korea
Non-idet P-40	Bio Basic Inc., Canada
Pfu DNA polymerase	Fermentas, Canada
Powder of Deoxy-Adenosine triphosphate	Fermentas, Canada
Powder of Deoxy-Cytidine triphosphate	Fermentas, Canada
Powder of Deoxy-Guanidine triphosphate	Fermentas, Canada
Powder of Deoxy-Thymidine triphosphate	Fermentas, Canada
Proteinase K	Bio Basic Inc., Canada
Taq DNA polymerase	Fermentas, Canada
Triton-X 100	Sigma-Aldrich Inc., Germany

Tris (anhydroxymethyl) aminomethane	Merck, Germany
SDS	Bio Basic Inc., Canada
Sodium acetate (CH <sub>3</sub> COONa)	Bio Basic Inc., Canada
Sodium chloride (NaCl)	Merck, Germany
<i>Xmn</i> I restriction enzyme	New England BioLabs Inc., USA
Xylene cyanol FF	Bio Basic Inc., Canada
<b>Oligonucleotide primers</b>	<b>Sources</b>
5'-GG-1	Qiagen GmbH, Germany
3'-AG-1	Qiagen GmbH, Germany
3.7 A	Proligo LLC, USA
3.7 B	Proligo LLC, USA
4.2 C	Proligo LLC, USA
4.2 D	Proligo LLC, USA
G I	Proligo LLC, USA
G II	Proligo LLC, USA

SEA-1	1 <sup>st</sup> BASE, Malaysia
SEA-2	1 <sup>st</sup> BASE, Malaysia
SEA-3	1 <sup>st</sup> BASE, Malaysia
$\alpha$ -AF	Bioservice Unit, Bangkok
$\alpha$ -BF	Bioservice Unit, Bangkok
$\alpha$ -F	Bioservice Unit, Bangkok
$\alpha_1$ -R	1 <sup>st</sup> BASE, Malaysia
$\alpha_2$ -R	Bioservice Unit, Bangkok
Beta-common-multiplex	1 <sup>st</sup> BASE, Malaysia
Beta-17-multiplex	1 <sup>st</sup> BASE, Malaysia
Beta-E-multiplex	1 <sup>st</sup> BASE, Malaysia
Beta-cds41/42-multiplex	1 <sup>st</sup> BASE, Malaysia

SEA-1-multiplex 1<sup>st</sup> BASE, Malaysia

SEA-2-multiplex 1<sup>st</sup> BASE, Malaysia

SEA-3-multiplex 1<sup>st</sup> BASE, Malaysia

## Appendix B

### List of instrument

#### Instruments

ABI Prism 3130 Genetic Analyzer

Analytical balance

Electrophoresis apparatus

Fume hood

Gel documentation system-100

Microcentrifuge tube (1.5 ml)

PCR machine (MJ research PTC-200)

PCR machine (Mycycler)

Pipette (max.vol. 10  $\mu$ l)

Pipette (max.vol. 20  $\mu$ l)

#### Sources

Applied Biosystems, USA

OHAUS Corp., USA

Bio 101, Inc., USA

Captair, France

Bio-rad, Co. Ltd, USA

Labcon Noth America, USA

Bio-rad, Co. Ltd, USA

Bio-rad, Co. Ltd, USA

HTC Lab Solution, Poland

HTC Lab Solution, Poland

Pipette (max.vol. 200 $\mu$ l)	HTC Lab Solution, Poland
Pipette (max.vol. 1000 $\mu$ l)	HTC Lab Solution, Poland
Power supply	Bio 101, Inc., USA
Refrigerated centrifuge	Thermo Electron, Germany
Spectrophotometry	Biochrom Ltd., England
Thin wall PCR tube (0.2 ml)	Labcon Noth America, USA
Thin wall PCR tube (0.5 ml)	Labcon Noth America, USA
Tip for pipette (for 0.5-10 $\mu$ l)	Labcon Noth America, USA
Tip for pipette (for 2-200 $\mu$ l)	Labcon Noth America, USA
Tip for pipette (for 100-1000 $\mu$ l)	Labcon Noth America, USA
Ultraviolet transilluminator	Spectronics Corp., USA
Waterbath	NÜVE, Ankara

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
 Copyright© by Chiang Mai University  
 All rights reserved

## Appendix C

### Reagent Preparation

#### 1. Agarose gel

Agarose gel were prepared in 0.5xTBE (%w/v)

#### 2. ANE buffer

Containing: 3 M NaOAc 0.5 ml

10% SDS 7.5 ml

0.5 M EDTA 0.3 ml

5 M NaCl 3.0 ml

Sterile distilled water 18.7 ml

This solution was stored at room temperature.



## 3. dNTPs

1 mM each of dNTPs: 10  $\mu$ l each of 100 mM dNTP was pooled and 960  $\mu$ l DW added before use.

## 4. 0.5 M EDTA (MW 292.25)

Containing:	EDTA	73.06 g
	Sterile distilled water	500 ml

This chemical was dissolved in 400-ml sterile distilled water. Then, adjust volume to 500 ml with sterile distilled water and stored at room temperature.

## 5. Ethidium bromide solution

Containing:	10 mg/ml Ethidium bromide	50 $\mu$ l
	1x TBE buffer	500 ml

This solution was mixed and stored at room temperature in light-protected bottle.

## 6. Loading buffer

Containing:	Ficoll type 400	15 g
	Bromophenol Blue	0.25 g
	Xylene cyanol FF	0.25 g
	5x TBE buffer	100 ml

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
Copyright © by Chiang Mai University  
All rights reserved

This chemical was mixed in 5x TBE buffer and stored in a glass bottle and stored at room temperature or  $-20^{\circ}\text{C}$  for longer period.

#### 7. Lysis buffer

Containing:	1 M Tris	0.5 ml
	5 M NaCl	0.1 ml
	0.5 M EDTA	1.0 ml
	Sterile distilled water	48.4 ml

This solution was mixed and stored in a centrifuge tube and stored at room temperature.

#### 8. 5 M NaCl (MW 55.44)

Containing:	Tris	146.1 g
	Sterile distilled water	500 ml

This chemical was dissolved in 400-ml sterile distilled water. Then, sterile distilled water were added to adjust volume to 500 ml and stored at room temperature.

#### 9. 0.5% NP-40

Containing:	NP-40	2.5 ml
	Sterile distilled water	500 ml

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

Copyright © by Chiang Mai University

All rights reserved

This solution was dissolved in 450-ml sterile distilled water. Then, sterile distilled water were added to adjust volume to 500 ml, slightly mix and stored at room temperature.

10. Proteinase K (20 mg/ml DW)

Containing:	Proteinase K	20 mg
	Sterile distilled water	100 ml

This chemical was dissolved in 95-ml sterile distilled water. Then, sterile distilled water were added to adjust volume to 100 ml and stored at room temperature.

11. 10% SDS

Containing:	SDS	5 g
	Sterile distilled water	50 ml

This chemical was dissolved in 45-ml sterile distilled water. Then, sterile distilled water were added to adjust volume to 50 ml and stored at room temperature.

12. 3 M Sodium acetate (  $\text{CH}_3\text{COONa}$  MW 82.03)

Containing:	$\text{CH}_3\text{COONa}$	246.09 g
	Sterile distilled water	500 ml

This chemical was dissolved in 400-ml sterile distilled water. Then, sterile distilled water were added to adjust volume to 500 ml and stored at room temperature.

## 13. 5x TBE buffer

Containing:	Tris (anhydroxymethyl) aminomethane	54.0 g
	Boric acid	27.5 g
	EDTA	4.75 g
	Distilled water	1,000 ml

This chemical was mixed in distilled water and stored at room temperature in a glass bottle.

## 14. TE buffer

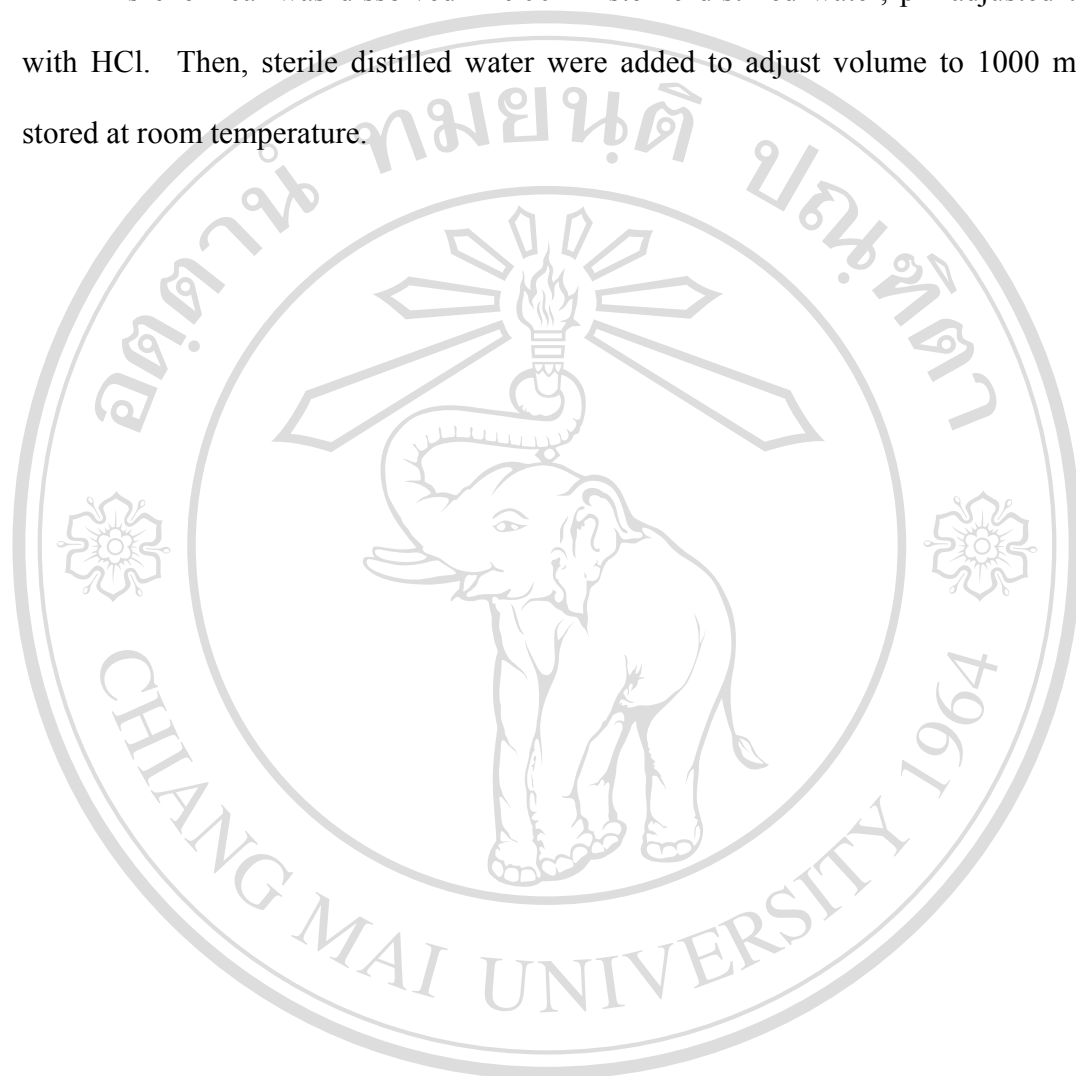
Containing:	1 M Tris	0.5 ml
	0.5 M EDTA	1 ml
	Sterile distilled water	48.5 ml

This solution was dissolved in 48.5-ml sterile distilled water and stored at room temperature.

## 15. 1 M Tris pH 8.0 (MW 121.1)

Containing:	Tris	121.1 g
	Sterile distilled water	1000 ml

This chemical was dissolved in 900-ml sterile distilled water, pH adjusted to 8.0 with HCl. Then, sterile distilled water were added to adjust volume to 1000 ml and stored at room temperature.



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

Copyright© by Chiang Mai University

All rights reserved

**CURRICULUM VITAE**

Name : Mr. Surasit suwannasin

Date of Birth : July 18, 1983

Place of Birth : Bangkok Province, Thailand

Education : Bachelor of Science (Medical Technology) Rangsit University,  
Pathum Thani, Thailand (2002-2005)

Address : 6/12 Ramkhamheang 128, Sukapibarn 3 Rd.,  
Saparnsung District, Bangkok Province 10240.

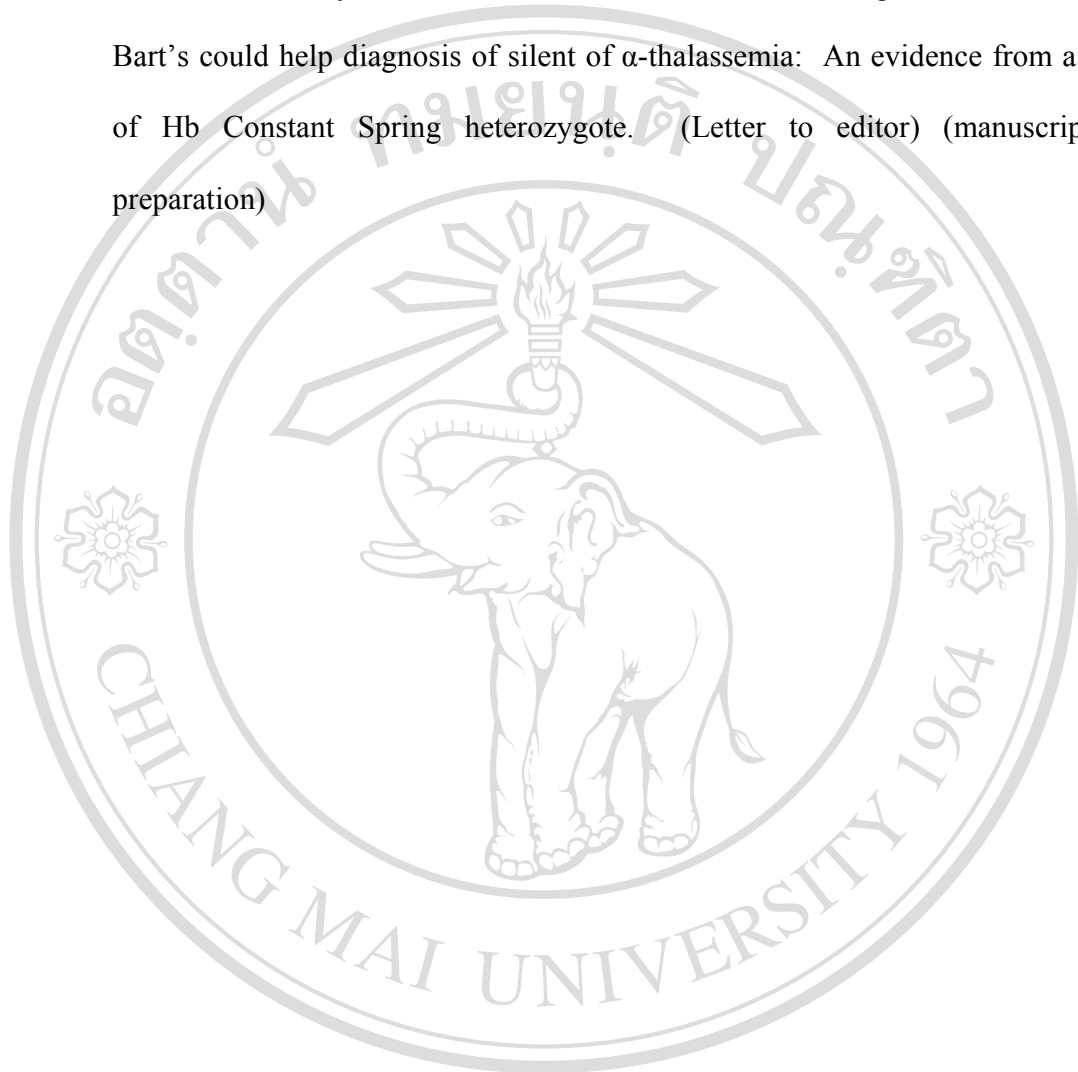
Tel. 0-2373-9533

## Output of thesis

1. Suwannasin S, Tatu T. Study of molecular background of silent  $\alpha$ -thalassemia.

Poster presentation at the year-2008, Annual Meeting of the Faculty of Associated Medical Sciences. Chiangmai University, at Lotus Pang Suan Kaew Hotel, Chiangmai, Dec 2<sup>nd</sup>-4<sup>th</sup>, 2008.

2. Suwannasin S, Sayachak S, Kasinrerak W, Tatu T. Immunological detection of Hb Bart's could help diagnosis of silent of  $\alpha$ -thalassemia: An evidence from a case of Hb Constant Spring heterozygote. (Letter to editor) (manuscript in preparation)



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
Copyright© by Chiang Mai University  
All rights reserved