CHAPTER VII

APPENDIX

APPENDIX A

List of chemicals

Chemicals	Sources
Absolute ethanol	Merck
Acetone	Merck
Agarose	Sigma Chemical Co. Ltd., USA
Anti-γ-globin	Gift kindly provided by Professor Swee Lay Thein of the Molecular Hematology Unit GKT School of Medicine King's Collage London, UK
Boric acid	Sigma Chemical Co. Ltd., USA
Chelex-100 (iminodiacetic acid)	Sigma Chemical Co. Ltd., USA
Distilled water (sterile)	Maharaj Nakorn Chiang Mai, Hospital Chiang Mai, Thailand
Ethylene diamine tetraacetate (disodium salts)	Sigma Chemical Co. Ltd., USA
Ethanol	BDH Laboratory Supplies, England
Ethidium bromide	Sigma Chemical Co. Ltd., USA

Ficoll 400	Pharmacia Ltd., Sweden
Glycerol	Merk
Magnesium chloride	QIAGEN Ltd., Germany
Methanol	BDH Laboratory Supplies, England
New Methylene Blue N solution	Sigma Chemical Co. Ltd., USA
Phosphate buffer (PBS)	Sigma Chemical Co. Ltd., USA
Sodium acetate	Merck
Trypsin	Sigma Chemical Co. Ltd., USA
Triton-X100	Sigma Chemical Co. Ltd., USA
Tri-hydroxy methyaminomethane	Sigma Chemical Co. Ltd., USA

Deoxynucleotides triphosphates

Powder of Deoxy-Adenosine triphosphate	QIAGEN Ltd., Germany
Powder of Deoxy-cytidine triphosphate	QIAGEN Ltd., Germany
Powder of Deoxy-Guanidine triphosphate	QIAGEN Ltd., Germany
Powder of Deoxy-Thymidine triphosphate	QIAGEN Ltd., Germany
DNA polymerase	
Taq DNA polymerase	QIAGEN Ltd., Germany

Oligonucleotide primers

5'-GG-1	Invitrogen, USA
3'-AG-1	Invitrogen, USA
5'-AG-5	QIAGEN Ltd., Germany
3'-AG-5	QIAGEN Ltd., Germany
P1-SEA	Finnzyme, Findland

P2-SEA	Finnzyme, Findland
P3-SEA	Finnzyme, Findland
N-41	Invitrogen, USA
M-41	Invitrogen, USA
C-41	Finnzyme, Findland
N-17	Finnzyme, Findland
M-17	Invitrogen, USA
C-17	Finnzyme, Finland
N-71/72	Invitrogen, USA
M-71/72	Finnzyme, Finland
C-71/72	Finnzyme, Finland
N-NT-28	Finnzyme, Finland
M-NT-28	Finnzyme, Finland
C-NT-28	Invitrogen, USA
N-654	Invitrogen, USA
M-654	Finnzyme, Finland
C-654	Finnzyme, Finland
N-E	Finnzyme, Finland
C-E	Finnzyme, Finland
M-E	Finnzyme, Finland

Restriction enzyme

*Xmn*I Restriction enzyme	Biolab., USA
ϕX174 DNA/Hae III Markers	Promega, USA

APPENDIX B

List of instruments

Instruments	Sources
Pipetman (max. vol. 10 µl)	Gilson, France
Pipetman (max. vol. 20 µl)	Gilson, France
Pipetman (max. vol. 100 µl)	Gilson, France
Pipetman (max. vol. 200 µl)	Gilson, France
Pipetman (max. vol. 1000 µl)	Gilson, France
ABI Prism 310 Genetic Analyzer	Perkin Elmer Cetus, USA
Analytical balance	Satorious, Germany
Blue tip for pipetman (for 1000 µl)	Treff-Swtzerland
Heating box	Eppendorf, Germany
Electrophoresis apparatus	Bio-rad, Co. Ltd., USA
Gel Doc 1000	Bio-rad, Co. Ltd., USA
High speed refrigerated centrifuge	Italy
Long wavelength UV	Supertonic Co. Ltd., NY
Microcentrifuge tube (1.5 ml)	Treff-Swtzerland
Microcentrifuge	Italy
PCR machine (system 2400)	Perkin Elmer Cetus, USA
PCR machine (system 9700)	Perkin Elmer Cetus, USA
PCR machine	Eppendorf, Germany
Power supply	C.B.S. Scientific, CA
Thinwall PCR tubes (0.2 ml)	Scientific Co. Ltd., CA

White tip for pipetman (for 10 µl)	Axygen, Inc., USA
Yellow tip for pipetman (for 20-200 µl)	Axygen, Inc., USA
Light microscopy	Olympus, USA
Fluorescence microscope	Olympus, USA

APPENDIX C

Reagent preparation

1. **Agarose gels** were prepared in 0.5X TBE
2. **1 mM each of dNTPs** : 10 µl each of 100 mM dNTP was pooled and 960 µl DW added before use.
3. **Ethidium bromide solution**

   Containing:

10 mg/ml Ethidium bromide	7.0	µl
1X TBE	75.0	ml
Distilled water	75.0	ml

   The solution was mixed and stored in a glass tray that was conserved with aluminium foil for protection from light and stored at room temperature.

4. **Loading buffer**

   Containing:

Ficoll (type 400)	15	g
Distilled water	100.0	ml

   Orange G was added to make color and stored at room temperature.

5. **Lysis buffer**

   Containing:

   0.5% Triton-X 100

   The solution was stored at 4°C.

6. **Primer stock solution dissolved in water**:

Each stock solution of primers were stored at –20°C. Working solution were prepared in 50 µl aliquots and stored at –20°C.

7. **Anti-γ-globin chain**:

A 1/100 dilution was prepared from stock solution containing FITC-conjugated anti-human γ-globin chain using 0.1% BSA in PBS as diluent.

8. **Trypsin solution**:

A trypsin tablet (Sigma T-7168 containing 1 mg porcine trypsin) was dissolved in 1 ml DW to prepare a 1 mg/ml trypsin solution and store for several months in a 10 µl (0.01 ng) aliquots. Working solution of 0.20 mg/ml was freshly prepared and warmed at 37°C before used.

9. **1 X Tris-borate ethylenediamine tetraacetate (TBE) solution, pH 8.3**

Stock 10X TBE

Containing:

89 M Tris-base

89 M H_3BO_3

24.9 mM EDTA

APPENDIX D

Clinical and hematological pictures of the patients with homozygous and compound heterozygous of β-thalassemia mutations analyzed in the present study

No.	β-thalassemia mutations	Hb (g/dl)	SEA	*Xmn*-I site	Hb typing	Blood Trans.	Age of onset (months)	Spleno-megaly	Hepato-megaly	Clinical diagnosis
1	$\beta^{41/42}/\beta^{41/42}$	3.0	N	-/-	A_2FA	11	6	Present	Present	TM
2	$\beta^{IVS1}/\beta^{IVS1}$	9.7	N	+/+	A_2FA	10	3.2	Present	Present	TM
3	$\beta^{41/42}/\beta^{17}$	7.4	N	+/-	A_2F	5	ND	Present	Present	TI
4	β^{17}/β^{NT-28}	4.4	N	-/-	A_2FA	11	20	Present	Present	TM
5	$\beta^{41/42}/\beta^{17}$	5.8	N	-/-	A_2FA	12	9	Present	Present	TM
6	$\beta^{41/42}/\beta^{41/42}$	6.6	N	+/-	A_2F	7	20	Present	Present	TM
7	$\beta^{41/42}/\beta^{17}$	7.6	N	+/-	A_2F	5	ND	Present	Present	TI
8	$\beta^{41/42}/\beta^{17}$	4.7	N	-/-	A_2FA	12	9	Present	Present	TM
9	$\beta^{41/42}/\beta^{27/28}$	6	N	-/-	A_2FA	12	17	Present	Present	TM
10	$\beta^{41/42}/\beta^{17}$	6.4	N	-/-	A_2F	3	12	Present	Present	TM
11	β^{17}/β^{NT-28}	9.8	N	+/-	A_2F	1	6	Present	Present	TI
12	$\beta^{IVS1}/\beta^{IVS1}$	7	N	-/-	A_2FA	7	ND	Present	Present	TM
13	$\beta^{IVS1}/\beta^{NT-87}$	7.9	N	-/-	A_2F	3	ND	Present	Present	TI
14	$\beta^{41/42}/\beta^{17}$	7.9	N	+/-	A_2FA	10	54	Present	Present	TM
15	$\beta^{41/42}/\beta^{41/42}$	5.7	N	-/-	A_2FA	12	ND	Present	Present	TM
16	$\beta^{41/42}/\beta^{41/42}$	4.2	N	-/-	A_2FA	10	ND	Present	Present	TM
17	$\beta^{41/42}/\beta^{41/42}$	6	N	-/-	A_2FA	12	ND	Present	Present	TM
18	$\beta^{41/42}/\beta^{17}$	6.8	P	-/-	A_2FA	12	23	Sp	Present	TM
19	β^{17}/β^{17}	5.8	N	-/-	A_2FA	13	14	Present	Present	TM
20	$\beta^{41/42}/\beta^{17}$	6.6	N	-/-	A_2FA	12	10	Present	Present	TM
21	β^{17}/β^{NT-28}	5.8	P	-/-	A_2FA	7	ND	Present	Present	TM
22	β^{17}/β^{17}	6.6	N	-/-	A_2FA	11	8	Present	Present	TM
23	$\beta^{41/42}/\beta^{41/42}$	6.2	N	-/-	A_2FA	14	17	Present	Present	TM

24	$\beta^{41/42}/\beta^{NT-28}$	7.5	N	+/-	A_2F	2	ND	Present	Present	TI
25	$\beta^{41/42}/\beta^{NT-28}$	5.2	N	+/-	A_2FA	8	ND	Present	Present	TM
26	$\beta^{IVS1}/\beta^{IVS1}$	9.5	N	+/+	A_2FA	10	38	Present	Present	TM
27	$\beta^{41/42}/\beta^{17}$	3	N	-/-	A_2FA	8	3	Present	Present	TM
28	$\beta^{41/42}/\beta^{41/42}$	6.4	N	-/-	A_2FA	15	31	Present	Present	TM
29	$\beta^{41/42}/\beta^{43}$	6	N	+/+	A_2F	0	44	Present	Present	TM
30	$\beta^{41/42}/\beta^{17}$	6	N	-/-	A_2FA	8	ND	Present	Present	TM

P = positive for SEA deletion type

N = negative for SEA deletion type

Sp = post splenectomy

TM = β-thalassemia major

TI = β-thalassemia intermedia

ND = not diagnosis

APPENDIX E

Clinical and hematological pictures of the patients with β-thalassemia/HbE disease analyzed in the present study

No.	β-thalassemia mutations	Hb (g/dl)	SEA	*Xmn*- I site	Hb typing	Blood Trans.	Age of onset (months)	Spleno-megaly	Hepato-megaly	Clinical diagnosis
1	β^{17}/β^{E}	6.5	N	-/-	EF	0	23	Present	Present	TI
2	$\beta^{41/42}/\beta^{E}$	3.3	N	+/-	EFA	12	3	Present	Present	TM
3	$\beta^{41/42}/\beta^{E}$	6.6	N	+/-	EFA	10	40	Present	Present	TM
4	$\beta^{41/42}/\beta^{E}$	6.0	N	-/-	EF	0	ND	Present	Present	TI
5	$\beta^{41/42}/\beta^{E}$	6.5	N	+/-	EF	0	36	Present	Present	TI
6	$\beta^{41/42}/\beta^{E}$	6.7	N	+/-	EF	0	2	Present	Present	TI
7	β^{NT-28}/β^{E}	7.0	N	+/-	EF	0	ND	Absent	Absent	TI
8	β^{NT-28}/β^{E}	5.4	N	+/-	EFA	2	ND	Absent	Absent	TI
9	β^{17}/β^{E}	5.6	N	+/-	EF	2	40	Present	Present	TI
10	$\beta^{41/42}/\beta^{E}$	6.8	N	-/-	EF	0	ND	Present	Present	TI
11	$\beta^{41/42}/\beta^{E}$	7.4	N	-/-	EF	0	9	Present	Present	TI
12	$\beta^{41/42}/\beta^{E}$	6.2	N	+/-	EF	5	38	Sp	Present	TM
13	$\beta^{41/42}/\beta^{E}$	7.7	N	+/-	EFA	2	0	Present	Present	TI
14	$\beta^{41/42}/\beta^{E}$	5.7	N	-/-	EF	4	6	Present	Present	TI
15	$\beta^{41/42}/\beta^{E}$	9.6	N	+/-	EF	0	ND	Absent	Absent	TI
16	$\beta^{41/42}/\beta^{E}$	7.4	N	+/-	EFA	2	3.8	Present	Present	TI
17	$\beta^{41/42}/\beta^{E}$	5.7	N	+/-	EFA	2	18	Sp	Present	TM
18	β^{NT-28}/β^{E}	9.1	N	+/-	EF	0	ND	Absent	Absent	TI
19	$\beta^{41/42}/\beta^{E}$	6.2	N	+/-	EFA	4	42	Sp	Present	TM
20	$\beta^{41/42}/\beta^{E}$	7.0	N	+/-	EF	0	ND	Present	Present	TI
21	$\beta^{41/42}/\beta^{E}$	9.2	N	+/-	EF	13	3	Absent	Absent	TI
22	$\beta^{41/42}/\beta^{E}$	6.9	P	+/-	EF	0	ND	Present	Present	TM

23	$\beta^{41/42}/\beta^{E}$	7.8	N	+/-	EF	2	ND	Present	Present	TI
24	$\beta^{41/42}/\beta^{E}$	5.6	N	+/-	EF	2	40	Present	Present	TI
25	$\beta^{41/42}/\beta^{E}$	6.7	N	-/-	EF	2	60	Present	Present	TI
26	β^{17}/β^{E}	6.5	N	-/-	EF	0	118	Present	Present	TI
27	$\beta^{41/42}/\beta^{E}$	9.2	N	+/-	EF	0	65	Present	Present	TI
28	β^{17}/β^{E}	6	N	-/-	EFA	7	38	Present	Present	TM
29	$\beta^{41/42}/\beta^{E}$	7.2	N	+/+	EF	0	ND	Present	Present	TI
30	$\beta^{41/42}/\beta^{E}$	ND	N	+/-	EF	0	48	Present	Present	TI

P = positive for SEA deletion type

N = negative for SEA deletion type

Sp = post splenectomy

TM = β-thalassemia major

TI = β-thalassemia intermedia

ND = not diagnosis

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