CHAPTER VII

APPENDIX

APPENDIX A

List of chemicals

Chemicals	Sources
Absolute ethanol	Merck
Agarose	Sigma Chemical Co. Ltd., USA
Boric acid	Sigma Chemical Co. Ltd., USA
Chelex-100 (iminodiacetic acid)	Sigma Chemical Co. Ltd., USA
Distilled water (sterile)	Faculty of Associated Medical Science , Chiang Mai Univercity
ϕ X 174 DNA/Hae III Marker	Promega, USA
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	Fermentas, USA
Triton-X 100	Sigma Chemical Co. Ltd., USA
Deoxynuclotide triphosphates	
Powder of Deoxy-Adenosin triphosphate	QIAGEN, GmbH, Germany
Powder of Deoxy-Cytidine triphosphate	QIAGEN, GmbH, Germany
Powder of Deoxy-Guanidine triphosphate	QIAGEN, GmbH, Germany
Powder of Deoxy-Thymidine triphosphate	QIAGEN, GmbH, Germany
Taq DNA polymerase	Fermentas, USA

Oligonucleotide primers

P1-SEA	INVITROGEN Ltd., Brazil
P2-SEA	INVITROGEN Ltd., Brazil
S	QIAGEN, GmbH, Germany
N-17	QIAGEN, GmbH, Germany
M-17	QIAGEN, GmbH, Germany
N-41/42	QIAGEN, GmbH, Germany
M-41/42	QIAGEN, GmbH, Germany
N-71/72	QIAGEN, GmbH, Germany
M-71/72	QIAGEN, GmbH, Germany
H63DR	INVITROGEN Ltd., Brazil
H63DF	INVITROGEN Ltd., Brazil
C282YR	INVITROGEN Ltd., Brazil
C282YF	INVITROGEN Ltd., Brazil
*Mbo*I	Fermentas, USA
*Rsa*I	Fermentas, USA
Buffer R with BSA	Fermentas, 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
Analytical balance	Satorious, Germany
Blue tip for pipetman (for 1000 µl)	Treff-Sweetzerland
Electrophoresis apparatus	Bio-rad, Co. Ltd., USA
Long wavelength UV	Supertonic Co. Ltd., NY
Microcentrifuge tube (1.5 ml)	Treff-Sweetzerland
Power supply	C.B.S. Scientific, CA
Thin wall 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

APPENDIX C

Reagents preparation

- **Mixed acid reagent** (1 mol HCl, 0.6 mol trichloroacetic acid and 0.4 mol thioglycollic acid per litre)
  1. Dissolve 98 g trichloracetic acid in 300 ml iron free water in a 1 litre volumetric flask
  2. Add 28 ml thioglycollic acid and 500 ml hydrochloric (2 mol/litre)
  3. Make to volume with iron-free water
  4. The solution is stable for at least 3 months if store in a dark brown bottle
- **Chromogen solution** (1.5 mol sodium acetate and 0.5 mmol bathophenanthroline sulphonate per litre)
  1. Dissolve 204 g sodium acetate trihydrate in 900 ml iron-free water
  2. Add 0.268 g bathophenanthroline sulphonate 4.:7-diphenly-1:10-phenanthroline) dissolved in 50 ml iron-free water
  3. Make to volume with iron-free water in a 1 litre volumetric flask
  4. The reagent is stable for at lest 2 weeks if kept in a dark brown bottle
- **Stock ferric chloride solution** (1 mol iron/litre and 50 mmol HCl/litre)

1. Dissolve 0.27 g ferric chloride ($FeCl_3.6H_2O$) in 50 ml HCl (1 mol/litre)
2. Make to volume with iron-free water in a 1 litre volumetric flask
3. The reagent is stable indefinitely and is used to prepare the iron-saturating solution for serum TIBC assay.

- **Saturating iron solution** (100 µmol iron/litre in 5 mmol HCl/litre). 10 ml stock ferric chloride solution made to 100 ml with iron-free water in a volumetric flask.
- **Light magnesium carbonate, reagent grade** (approximate formula $3MgCO_3$, $Mg(OH)_2$, $3H_2O$)
- **Working iron standard** (100 mg/dl)

  Dilute stock iron standard 100,000 mg/dl to 100 mg/dl by deionized water
- **10 x TBE : 5 litres**

Containing : 540 g Tris

275 g boric acid

47.5 g EDTA

- **Agarose gels** were prepare in 1 x TBE
- **Lysis buffer**

  Containing : 10 mM Tris pH 8

  10 mM NaCl

  10 mM EDTA
- **Acetate-NaCl-EDTA (ANE) buffer (pH 6.0)**

  Containing : 0.05 M $CH_3COONa.3H_2O$

  05M NaCl

  0.005M EDTA

  2.5% SDS
- **1 mM each of dNTPs** : 10 µl of 100 mM dNTP was pooled and 960 µl DW added before use.
- **Ethidium bromide**

  Containing : 10 mg/ml Ethidium bromide 15 µl

  1 x TBE 300 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.
- **Primer stock solution dissolved in water:**

  Each stock solution of primers were stored at -20°C. Working solution were prepared in 500 µl aliquots and stored at -20°C
- **Loading buffer**

  Containing :

Ficoll (type 400)	15	g
Distilled water	100.0	ml

  Bromophenolblue was added to make color and stored at room temperature.

APPENDIX D

The data of the patients with β-thalassemia/HbE analyzed in the present study

No.	Age (Yrs.)	RBC	Hb	Hct	MCV	MCH	MCHC	%A	%A_2	%F	Hb typing	ZPP	SI	TIBC	TS	β-thalassemia genotypes
1		3.28	9.89	19.1	58.2	16.8	28.8	3.7	62.5	24.9	EFA	42	88	192	46	cd17/HbE
2		3.07	5.5	21.6	70.4	17.9	25.5	3.2	75.2	15.2	EFA	147	214	257	83	cd17/HbE
3	28	3.08	7	23.7	76.9	22.7	29.5	2.8	46.4	41.4	EFA	82	80	244	33	cd17/HbE
4		4.57	8.5	28.1	61.5	18.6	30.2	2.7	60.6	29.2	EFA	35	99	230	43	cds41/42/HbE
5		2.67	5.6	23.6	88	21.1	23.9	2.5	57.2	30.5	EFA	79	162	208	78	cd17/HbE
6		3.35	6.3	21.2	63	18.8	29.7	2.1	50.1	25.2	EFA	66	130	156	83	cds41/42/HbE
7		2.57	6	24.5	96	23.2	24.3	2.6	65.4	22.2	EFA	104	202	204	99	cds41/42/HbE
8		3.02	5.5	21	70	18.2	26.2	3.5	72	15.9	EFA	106	125	160	78	cd17/HbE
9		3.34	6.6	25.7	77	19.6	25.5	2.5	59.8	27.2	EFA	111	109	139	78	cds41/42/HbE
10		3.42	7.5	25.5	74	21.8	29.3	1.7	40.7	47.4	EFA	86	168	174	96	cd17/HbE
11		2.32	5.4	20.8	89	23.3	26.1	1.7	47	38.9	EFA	86	194	236	82	cd17/HbE
12		5.22	14.8	42.8	82	28.3	34.5	3.2	65	22.4	EFA	96	159	323	49	cd17/HbE

No.	Age (Yrs.)	RBC	Hb	Hct	MCV	MCH	MCHC	%A	%A_2	%F	Hb typing	ZPP	SI	TIBC	TS	β-thalassemia genotypes
13		4.94	14.3	43.4	88	29.2	33	3.1	76.7	12.2	EFA	56	99	116	85	?/HbE
14		3.97	8.4	27	68	21.2	31.1	2.5	48.6	38.8	EFA	59	169	158	107	?/HbE
15	25	4	6	18	70	18.2	26	16.8	47.5	26.2	AFE	87	177	272	65	cds41/42/HbE
16	21	3.34	6.5	25.7	72	19.5	25.5	9.2	65.7	17	AFE	69	257	269	96	cds41/42/HbE
17	19	4.25	9	30	56.2	17.1	29.7	9	69.4	13.7	AFE	82	234	232	101	cd17/HbE
18	32	3.4	5.5	19	58.2	16.8	28.8	42.4	43.1	5.7	AFE	66	214	284	75	cds41/42/HbE
19	5	2.28	4.6	15.9	69.7	20.2	28.9	44.6	32.3	11.6	AFE	80	220	180	122	cd17/HbE
20	10	3.54	7	23.6	66.7	19.8	29.7	39	34	18.1	AFE	62	176	184	96	cds41/42/HbE
21	37	4.28	7.6	26.3	61.4	17.8	28.9	2.4	50.6	34.8	AFE	87	203	230	88	cd17/HbE
22	21	3.79	7.6	25.4	67	20.1	29.9	2	52	37.9	AFE	76	301	189	159	cds41/42/HbE
23	36	4.15	7.5	27.1	65.3	18.1	27.7	2.8	65.8	22.2	AFE	104	148	241	61	cd17/HbE
24	29	5.07	8.3	28.3	55.8	16.4	23.7	2.8	83.1	8.3	AFE	56	148	209	71	cds41/42/HbE
25	20	4.14	7	24.2	58.5	16.9	28.9	2.8	78.5	12.7	AFE	42	145	184	79	cds41/42/HbE
26	12	3.31	5.2	17.8	53.8	15.7	29.2	8.1	62.9	20.1	EFA	202	68	246	28	cd17/HbE
27	32	2.66	5.6	19	71.4	21.1	29.5	2.2	46.1	39.4	AFE	57	170	263	65	cd17/HbE

No.	Age (Yrs.)	RBC	Hb	Hct	MCV	MCH	MCHC	%A	$\%A_2$	%F	Hb typing	ZPP	SI	TIBC	TS	β-thalassemia genotypes
28	21	3.58	6.2	21.3	59.5	17.3	29.1	10.8	59.5	22.4	AFE	80	143	158	91	cd17/HbE
29	25	3.82	9.1	31.1	81.4	23.8	29.3	1.6	37.6	46.7	AFE	97	134	233	58	ND
30	28	3.42	5.9	23.1	67.5	17.3	25.5	3	68.1	17.3	EFA	79	273	357	76	ND
31	43	3.6	8.6	27.2	75.6	23.9	31.6	1.3	36.3	47.9	EFA	64	277	302	92	ND
32	36	3.98	7	24.4	61.3	17.6	28.7	1.5	48.4	37.4	EFA	52	169	220	77	cd17/HbE
33	23	4.3	7	23.7	55.1	16.3	29.5	3.3	58.5	22.9	AFE	86	190	394	48	cd17/HbE
34	32	3.43	6.4	22.8	66.5	18.7	28.1	1.3	44.3	38.6	EFA	53	561	787	71	cd17/HbE

ND: Not done

? : Negative for cds41/42 and cd17

APPENDIX E

The data of the patients with homozygous β-thalassemia analyzed in the present study

No.	Age (Yrs.)	RBC	Hb	Hct	MCV	MCH	MCHC	%A	%A_2	%F	Hb typing	ZPP	SI	TIBC	TS	β-thalassemia genotypes
1		2.35	6	19	80.9	25.5	31.6	76.2	3.4	8.7	A_2AF	52	248	226	110	cds41/42/?
2		4.75	10.6	33.9	71.4	22.3	31.3	82.4	5.9	0.9	A_2AF	50	129	212	61	cd17/?
3		5.26	9	30.3	57.6	17.1	29.7	84.5	5.2	1.2	A_2AF	132	129	260	50	cd17/?
4		2.04	4.9	17.3	84	24.2	28.7	74.6	3.2	8.2	A_2AF	64	251	267	94	cd17/cds41/42
5		3.16	7.1	23.9	75.6	22.5	29.7	33.1	4.5	45.9	A_2AF	53	188	221	85	cd17/cds41/42
6	17	4.5	8.4	28	60.2	18.5	30.5	26.7	6.4	53	A_2AF	53	226	241	94	cd17/?
7	13	2.64	6.6	21.5	81.4	25	30.7	65.2	3.5	15.7	A_2AF	53	147	169	87	cd17/?
8	12	2.23	5.6	18	80.7	25.1	31.1	33.9	2.1	52.2	A_2AF	71	148	150	99	?/?
9	14	1.57	4	133	84.7	25.5	30.1	44.4	3.6	38.8	A_2AF	75	204	264	77	?/?
10	17	2.21	6.1	19.4	87.8	27.6	31.4	70.5	4	10.9	A_2AF	65	331	217	153	cds41/42/ cds41/42
11	12	2.75	6.6	21	76.4	24	31.4	50.5	2.6	34.4	A_2AF	53	208	254	82	cd17/?
12	10	2.43	6.6	20.2	83.1	27.2	32.7	61.1	2.6	21.1	A_2AF	58	200	460	43	cds41/42/?

No.	Age (Yrs.)	RBC	Hb	Hct	MCV	MCH	MCHC	%A	%A_2	%F	Hb typing	ZPP	SI	TIBC	TS	β-thalassemia genotypes
13	5	2.45	6.5	18.6	75.9	26.5	34.9	79.3	2.4	6.8	A_2AF	50	137	246	56	cds41/42/?
14	25	2.64	6.6	22.5	85.2	25	29.3	53.2	4.3	32.2	A_2AF	69	387	435	89	ND
15	7	3.15	7.8	24	76.2	24.8	32.5	61.4	2.2	22.1	A_2AF	54	519	713	73	cd17/cds41/42
16	14	1.34	3.5	12.3	91.8	26.1	28.5	71.7	3.1	10.3	A_2AF	101	262	312	84	cd17/cds41/42
17	11	2.35	6	18.5	78.7	25.5	32.4	72	2.2	7.5	A_2AF	56	172	244	70	cd17/cds41/42
18	23	2.39	5	18.1	75.7	20.9	27.6	3.2	0.2	75.1	A_2AF	71	317	411	77	cd17/cd17
19	18	4.24	10.1	31	73.1	23.8	32.6	65.4	5.9	15.6	A_2AF	54	170	200	85	cds41/42/?
20	18	1.69	4.5	14.1	83.4	26.6	31.9	72.2	0.2	11.8	A_2AF	58	458	740	62	cd17/cds41/42
21	6	2.33	7.1	20	85.8	30.5	35.5	80	4.7	6.3	A_2AF	49	483	760	64	cd17/cd17
22	8	2.84	6.9	21.3	75	24.3	32.4	80.5	0.2	8.7	A_2AF	39	208	215	97	cd17/cds41/42
23	23	3.58	8	27.2	76	22.3	29.4	0.2	2.2	70.9	A_2AF	57	172	260	66	cd17/cds41/42

ND: Not done

? : Negative for cds41/42 and cd17

APPENDIX F

The data of the non- β-thalassemia analyzed in the present study

No	Age	RBC	Hb	Hct	MCV	MCH	MCHC	%A	%A2	Hb typing	ZPP	SI	TIBC	TS
1	21	4.93	11.6	36.7	74.4	23.1	31.1	85.9	2.8	A_2A	55	134	431	31
2	24	4.19	13.5	39.1	93.3	32.2	34.5	87.6	2.7	A_2A	26	131	367	35
3	46	3.68	11.6	34.2	92	31	33.3	73.8	1.2	A_2A	40	69	296	23
4	23	4.64	11.9	37.9	81	25.6	31.4	71.1	1.8	A_2A	61	78	261	30
5	60	4.42	13.8	42.1	95	31.2	32.8	73.8	0.2	A_2A	56	112	334	33
6	21	5.19	14	44.2	85	27	31.7	70.2	0	A_2A	28	130	300	43
7	53	4.53	13.6	42	92	30	32.4	73.2	1.0	A_2A	40	144	282	51
8	21	4.91	14.8	42.7	87	30.1	34.7	86.3	2.1	A_2A	18	99	285	35
9	19	6.09	13.4	43.7	71.8	22	30.7	86.1	1.7	A_2A	32	140	320	44
10	19	4.54	12.8	41.3	91	28.2	31	88.8	1.8	A_2A	20	130	360	36
11	22	4.73	13.8	42.8	90.5	29.2	32.2	86.8	2.1	A_2A	27	141	220	64
12	22	6.08	13.6	45	74	22.4	30.2	86.4	1.7	A_2A	27	109	187	58
13	22	4.22	12.5	38.3	90.8	29.6	32.6	85.4	1.4	A_2A	37	116	320	36

No	Age	RBC	Hb	Hct	MCV	MCH	MCHC	%A	%A2	Hb typing	ZPP	SI	TIBC	TS
14	33	4.84	14.5	43.9	90.7	30	33	86.4	1.5	A_2A	22	142	230	62
15	44	4.33	14.4	42.8	98.8	33.3	33.6	83.9	1.3	A_2A	42	170	230	74
16	19	4.75	14.6	43.7	92	30.7	33.4	87.8	2.2	A_2A	30	151	221	68
17	28	4.89	14.2	43.6	89.2	29	32.6	85.1	1.4	A_2A	22	108	217	50
18	20	3.96	12.3	37.2	93.9	31.1	33.1	88.4	1.5	A_2A	22	131	262	50
19	22	4.95	15.6	45.5	91.9	31.5	34.3	86.5	1.5	A_2A	40	119	215	55
20	35	4.64	14.7	44.8	96.6	31.7	32.8	90.9	1.5	A_2A	18	140	281	50
21	25	4.6	14.9	45	97.8	32.4	33.1	92.2	2.6	A_2A	33	111	205	54
22	54	5.35	14.9	45.9	85.8	27.9	32.5	84.5	1.1	A_2A	38	96	273	35

CURRICULUM VITAE

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