

CHAPTER VII

APPENDIX

APPENDIX A

List of chemicals

Chemicals

Absolute ethanol

Agarose

Boric acid

BigDye Terminator Sequencing Kit

Chelex-100 (iminodiacetic acid)

Distilled water (sterile)

100 bp DNA ladder

Ethylene diamine tetraacetate (disodium salts)

Ethanol

Ethidium bromide

310 Genetic Analyzer Buffer 10X

Ficoll 400

Glycerol

Sources

Merck, Germany

Sigma Chemical Co. Ltd., USA

Sigma Chemical Co. Ltd., USA

Perkin Elmer Co., NJ, USA

Sigma Chemical Co. Ltd., USA

Maharaj Nakorn Chiang Mai,

Hospital Chaing Mai, Thailand

New England BioLabs Inc., USA

Sigma Chemical Co. Ltd., USA

BDH Laboratory Supplies,

England

Sigma Chemical Co. Ltd., USA

Perkin Elmer Co., NJ, USA

Pharmacia Ltd., Sweden

Merck, Germany

HotStart <i>Taq</i> DNA polymerase	QIAGEN, GmbH, Germany
Magnesium chloride	QIAGEN, GmbH, Germany
Triton-X 100	Sigma Chemical Co. Ltd., USA
Tri-hydroxy methyaminomethane	Sigma Chemical Co. Ltd., USA
<u>Deoxynucleotide triphosphates</u>	
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
DNA polymerase	
<i>Taq</i> DNA polymerase	QIAGEN, GmbH, Germany
<u>Oligonucleotide primers</u>	
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
N-IVS	QIAGEN, GmbH, Germany
NC-IVS	QIAGEN, GmbH, Germany
M-IVS	QIAGEN, GmbH, Germany
Hb E-N	INVITROGEN Ltd., Brazil
Hb E-M	INVITROGEN Ltd., Brazil

APPENDIX B

List of instruments

<u>Instruments</u>	<u>Sources</u>
Pipetman (max. vol. 10 μ l)	Gilson, France
Pipetman (max. vol. 20 μ l)	Gilson, France
Pipetman (max. vol. 100 μ l)	Gilson, France
Pipetman (max. vol. 200 μ l)	Gilson, France
Pipetman (max. vol. 1000 μ l)	Gilson, France
ABI Prism 310 Genetic Analyzer	Perkin Elmer Cetus, USA
Analytical balance	Satorious, Germany
Blue tip for pipetman (for 1000 μ l)	Treff- Switzerland
Heating block	Eppendorf, Germany
Electrophoresis apparatus	Bio-rad, Co. Ltd., USA
Gel Doc 1000	Bio-rad, Co. Ltd., USA
Long wavelength UV	Supertonic Co. Ltd., NY
Microcentrifuge tube (1.5 ml)	Treff- Switzerland
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, USA
Thin wall PCR tubes (0.2 ml)	Scientific Co. Ltd., CA, USA
White tip for pipetman (for 10 μ l)	Axygen, Inc., USA
Yellow tip for pipetman (for 20-200 μ l)	Axygen, Inc., USA

APPENDIX C

Reagent preparation

1. Agarose gels were prepared in 0.5X TBE
2. 1 mM each of dNTPs : 10 μ l each of 100 mM dNTP was pooled and 960 μ l DW added before use.

3. Ethidium bromide solution

Containing:

10 mg/ml Ethidium bromide	15.0 μ l
0.5 x TBE	300.0 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^oC

6. Primer stock solution dissolved in water:

Each stock solution of primers were stored at -20^oC. Working solution were prepared in 100 μ l aliquots and stored at -20^oC.

7. 0.5X Tris-borate ethylenediamine tetraacetate (TBE) solution, pH 8.3

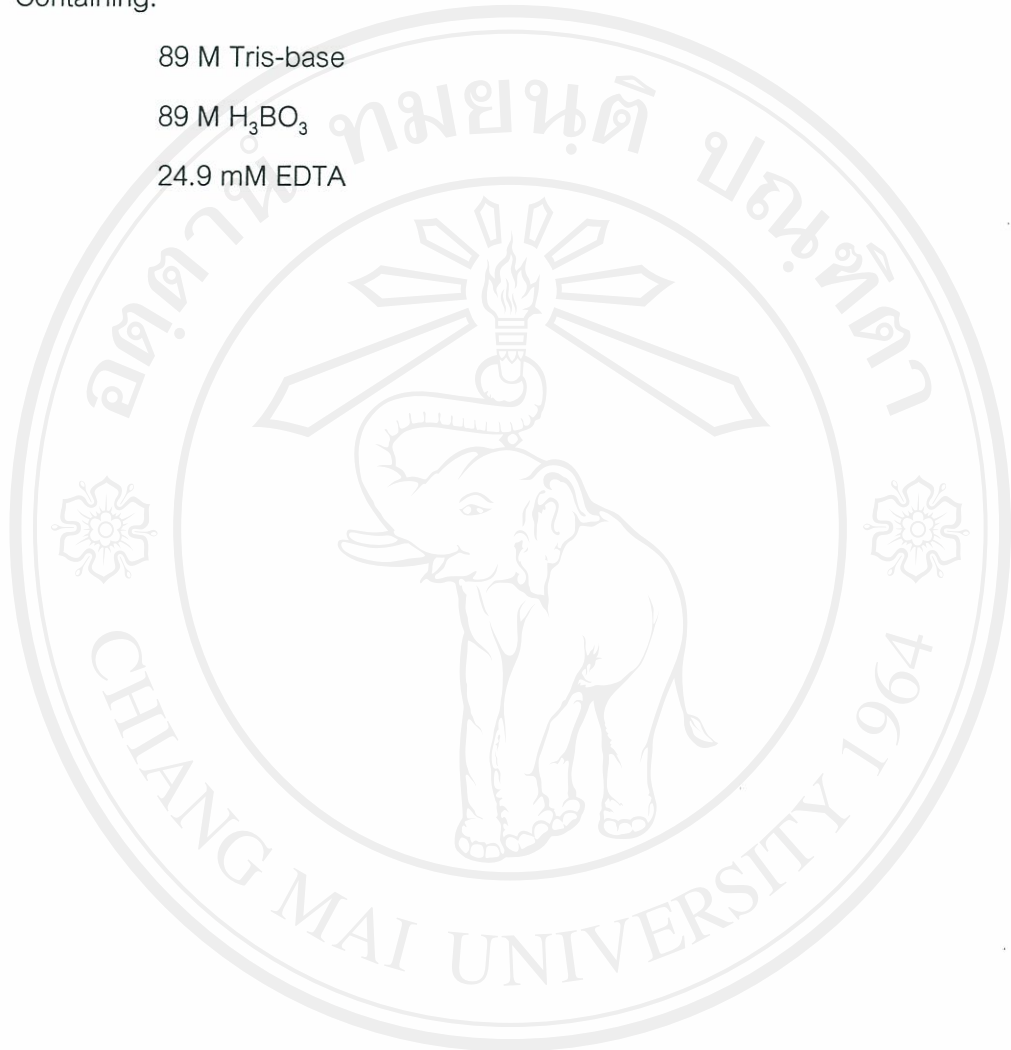
Stock 10 X TBE

Containing:

89 M Tris-base

89 M H_3BO_3

24.9 mM EDTA



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APPENDIX D

Raw data in β -thalassemia heterozygote screening

Neg = negative

Pos = positive

Het = heterozygote

ND = not done

1. Group I

No	OFT	%Hb A ₂ (HPLC)	SEA-PCR	ARMS-PCR			
				Cd 17	Cd41/42	Cd71/72	IVS Int1G-T
1	Neg	2.6	Neg	Neg	Neg	Neg	Neg
2	Neg	2.9	Neg	Neg	Neg	Neg	Neg
3	Neg	3.3	Neg	Neg	Neg	Neg	Neg
4	Neg	3.5	Neg	Neg	Neg	Neg	Neg
5	Neg	3	Neg	Neg	Neg	Neg	Neg
6	Neg	3.5	Neg	Neg	Neg	Neg	Neg
7	Neg	2.8	Neg	Neg	Neg	Neg	Neg
8	Neg	2.9	Neg	Neg	Neg	Neg	Neg
9	Neg	3.2	Neg	Neg	Neg	Neg	Neg
10	Neg	3	Neg	Neg	Neg	Neg	Neg
11	Neg	2.9	Neg	Neg	Neg	Neg	Neg
12	Neg	2.8	Neg	Neg	Neg	Neg	Neg
13	Neg	3.1	Neg	Neg	Neg	Neg	Neg
14	Neg	3.2	Neg	Neg	Neg	Neg	Neg
15	Neg	3.2	Neg	Neg	Neg	Neg	Neg

2. Group II

No	OFT	%Hb A ₂ (HPLC)	SEA-PCR	ARMS-PCR			
				Cd 17	Cd41/42	Cd71/72	IVS Int1G-T
1	Pos	3.4	Neg	Neg	Neg	Neg	Neg
2	Pos	3.2	Neg	Neg	Neg	Neg	Neg
3	Pos	2.9	Neg	Neg	Neg	Neg	Neg
4	Pos	3.1	Neg	Neg	Neg	Neg	Neg
5	Pos	2.9	Neg	Neg	Neg	Neg	Neg
6	Pos	2.9	Neg	Neg	Neg	Neg	Neg
7	Pos	2.9	Neg	Neg	Neg	Neg	Neg
8	Pos	3.1	Neg	Neg	Neg	Neg	Neg
9	Pos	2.4	Neg	Neg	Neg	Neg	Neg
10	Pos	3.1	Neg	Neg	Neg	Neg	Neg
11	Pos	3	Neg	Neg	Neg	Neg	Neg
12	Pos	2.9	Neg	Neg	Neg	Neg	Neg
13	Pos	2.6	Neg	Neg	Neg	Neg	Neg
14	Pos	3.8	Neg	Neg	Neg	Neg	Neg
15	Pos	2.6	Neg	Neg	Neg	Neg	Neg

3. Group III

No	OFT	%Hb A ₂ (HPLC)	SEA-PCR	ARMS-PCR			
				Cd 17	Cd41/42	Cd71/72	IVS Int1G-T
1	Pos	2.5	Pos	Neg	Neg	Neg	Neg
2	Pos	3.3	Pos	Neg	Neg	Neg	Neg
3	Pos	3.4	Pos	Neg	Neg	Neg	Neg
4	Pos	3.1	Pos	Neg	Neg	Neg	Neg
5	Pos	3.1	Pos	Neg	Neg	Neg	Neg
6	Pos	3.1	Pos	Neg	Neg	Neg	Neg
7	Pos	2.6	Pos	Neg	Neg	Neg	Neg
8	Pos	2.3	Pos	Neg	Neg	Neg	Neg
9	Pos	2.8	Pos	Neg	Neg	Neg	Neg
10	Pos	3.4	Pos	Neg	Neg	Neg	Neg
11	Pos	2.4	Pos	Neg	Neg	Neg	Neg
12	Pos	3.2	Pos	Neg	Neg	Neg	Neg
13	Pos	2.7	Pos	Neg	Neg	Neg	Neg
14	Pos	2.9	Pos	Neg	Neg	Neg	Neg
15	Pos	2.9	Pos	Neg	Neg	Neg	Neg

4. Group IV

No	OFT	%Hb A ₂ (HPLC)	SEA-PCR	ARMS-PCR			
				Cd 17	Cd41/42	Cd71/72	IVS Int1G-T
1	Pos	4.6	Neg	Het	Neg	Neg	Neg
2	Pos	5.3	Neg	Neg	Het	Neg	Neg
3	Pos	5.9	Neg	Neg	Het	Neg	Neg
4	Pos	5.5	Neg	Het	Neg	Neg	Neg
5	Pos	6.4	Neg	Neg	Neg	Het	Neg
6	Pos	5.7	Neg	Neg	Het	Neg	Neg
7	Pos	6	Neg	Neg	Neg	Neg	Neg
8	Pos	6	Neg	Neg	Het	Neg	Neg
9	Pos	6.3	Pos	Het	Neg	Neg	Neg
10	Pos	6	Neg	Het	Neg	Neg	Neg
11	Pos	4.9	Neg	Neg	Het	Neg	Neg
12	Pos	5	Pos	Neg	Neg	Neg	Het
13	Pos	6	Neg	Neg	Neg	Neg	Neg
14	Pos	7.1	Neg	Neg	Het	Neg	Neg
15	Pos	5.8	Neg	Het	Neg	Neg	Neg
16	Pos	5.6	Neg	Neg	Het	Neg	Neg
17	Pos	6.3	Neg	Neg	Het	Neg	Neg
18	Pos	6.2	Neg	Het	Neg	Neg	Neg
19	Pos	6.2	Neg	Neg	Het	Neg	Neg
20	Pos	3.8	Neg	Het	Neg	Neg	Neg
21	Pos	6	Neg	Neg	Het	Neg	Neg
22	Pos	6.4	Neg	Neg	Het	Neg	Neg

Group IV (continue)

No	OFT	%Hb A ₂ (HPLC)	SEA-PCR	ARMS-PCR			
				Cd 17	Cd41/42	Cd71/72	IVS Int1G-T
23	Pos	4.1	Neg	Het	Neg	Neg	Neg
24	Pos	5.6	Neg	Neg	Het	Neg	Neg
25	Pos	5.9	Neg	Neg	Het	Neg	Neg
26	Pos	6	ND	Neg	Het	Neg	Neg
27	Pos	5.7	Pos	Het	Neg	Neg	Neg
28	Pos	5.3	Neg	Neg	Het	Neg	Neg
29	Pos	5.8	Neg	Neg	Neg	Neg	Het
30	Pos	5.5	Neg	Neg	Het	Neg	Neg

APPENDIX E

Raw data in Hb E screening

Neg = negative

Pos = positive

Het = heterozygote

Homo = homozygote

ND = not done

No	OFT	%Hb A ₂ (HPLC)	SEA-PCR	ARMS-Hb E
1	Pos	23	Pos	Het
2	Neg	30.3	Neg	Het
3	Pos	32.8	Neg	Het
4	Neg	30.5	Neg	Het
5	Pos	32.1	Neg	Het
6	Pos	94.4	Neg	Homo
7	Pos	29.3	Neg	Het
8	Pos	82	Neg	Homo
9	Pos	32.3	Neg	Het
10	Pos	29.8	Neg	Het
11	Pos	32.7	Neg	Het
12	Pos	31.2	Neg	Het
13	ND	31.5	Neg	Het
14	ND	32.2	Neg	Het
15	Pos	32.6	Neg	Het
16	Pos	32.6	Neg	Het
17	Pos	31.5	Neg	Het

Continue

No	OFT	%Hb A ₂ (HPLC)	SEA-PCR	ARMS-Hb E
18	Pos	99.5	Neg	Homo
19	Pos	31.2	Neg	Het
20	Pos	29.5	Neg	Het
21	ND	91.8	Neg	Homo
22	ND	26.2	Neg	Het
23	ND	22.6	Neg	Het
24	ND	21.5	Neg	Het
25	Neg	20.6	Neg	Het
26	Pos	19	Neg	Het
27	Pos	18.4	Pos	Het
28	Pos	28.9	Pos	Het
29	ND	88.1	Neg	Homo
30	Pos	29.3	Neg	Het
31	Pos	24.4	Neg	Het
32	Pos	21.8	Neg	Het
33	Pos	23.5	Neg	Het
34	Pos	23.3	Neg	Het
35	Pos	26.1	Neg	Het
36	ND	98.6	Neg	Homo
37	Pos	27.4	Neg	Het
38	Pos	23.9	Neg	Het
39	Pos	28.3	Neg	Het
40	Pos	14.4	Pos	Het

Continue

No	OFT	%Hb A ₂ (HPLC)	SEA-PCR	ARMS-Hb E
41	ND	81.5	Pos	Homo
42	Pos	19.3	Neg	Het
43	Pos	26.4	Neg	Het
44	Pos	24.7	Neg	Het
45	ND	91.1	Neg	Homo
46	Pos	19.3	Neg	Het
47	Pos	26.8	Neg	Het
48	Pos	33.7	Neg	Het
49	Pos	25.7	Neg	Het
50	ND	88	Neg	Homo

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APPENDIX F

Raw data in fetal blood and parents at risk β -thalassemia major

Pos = Positive

Neg = Negative

ND = not done

Het = Heterozygote

Homo = Homozygote

Fam = Family

FB = fetal blood

CVS = chorionic villi sampling

M = mother

F = father

GA = gestation age

R = repeat

		PND Code	GA (Weeks)	HPLC	Diagnosis By HPLC	ARMS-PCR			
						Cd17	Cd41/42	Cd71/72	IVS I-nt 1 G-T
Fam 1	FB1	2208	20	A=4.2	Normal	Neg	Het @	Neg	Neg
	M1	168		A ₂ = 4.9		Het	Neg	Neg	ND
	F1	169		A ₂ = 5.4		Neg	Het	Neg	ND
Fam 2	FB2	2392	18	A=4.6	Normal	Neg	Neg	Neg	Neg
	M2	675		A ₂ = 4.5		Neg	Neg	Neg	Het
	F2	676		A ₂ =5.1		Neg	Neg	Neg	Het
Fam 3	FB3	2827	19	A=3.6	Normal	Neg	Neg	Neg	Neg
	M3	19/15		A ₂ =5.2		Neg	Het	Neg	ND
	F3	20/15		A ₂ =5.5		Het	Neg	Neg	ND

Continue.

	No	PND Code	GA (weeks)	HPLC	Diagnosis By HPLC	ARMS-PCR			
						Cd17	Cd41/42	Cd71/72	IVS 1 nt I G-T
Fam 4	FB4	2483	18	A=4.2	Normal	Neg	Het @	Neg	Neg
	M4	970		A ₂ =4.5		Neg	Neg	Neg	Het
	F4	971		A ₂ =6.2		Neg	Het	Neg	ND
Fam 5	FB5	2797	18	A=2.5	Normal	Het @	Neg	Neg	Neg
	M5	7/8		β -trait		Het	Neg	Neg	ND
	F5	8/8		β -trait		Neg	Het	Neg	ND
Fam 6	FB6	2605	19	A=0	β -thalassemia major	Het	Neg	Neg	Het
	M6	310		A ₂ =5.5		Neg	Neg	Neg	Het
	F6	311		A ₂ =4.5		Het	Neg	Neg	Neg
Fam 7	FB7	2795	18	A=3.5	Normal	Neg	Neg	Het@	Neg
	M7	15/19		A ₂ =5.9		Het	Neg	Neg	ND
	F7	16/19		A ₂ =5.9		Neg	Neg	Het	ND
Fam 8	FB8	2696	18	A = 3.2	β -thalassemia trait	Neg	Neg	Neg	Het
	M8	736		A ₂ =4.6		Neg	Neg	Neg	Het
	F8	737		A ₂ =4.6		Neg	Neg	Neg	Het
Fam 9	FB9	2845	20	A=0	β -thalassemia major	Neg	Homo	Neg	ND
	M9	539		A ₂ =5.9		Neg	Het	Neg	ND
	F9	540		A ₂ =4.9		Neg	Het	Neg	ND
Fam 10	FB10	3050	20	A=0	β -thalassemia major	Het	Het	Neg	ND
	M10	870		A ₂ =4.8		Het	Neg	Neg	ND
	F10	871		A ₂ =4.7		Neg	Het	Neg	ND

Continue.

	No	PND Code	GA (Weeks)	HPLC	Diagnosis By HPLC	ARMS-PCR			
						Cd17	Cd41/42	Cd71/72	IVS 1 nt I G-T
Fam 11	FB11	2944	21	A=14.2	Normal	Neg	Neg	Neg	Neg
	M11	597		A ₂ =5.1		Neg	Neg	Neg	Het
	F11	20/8		A ₂ =5.2		Het	Neg	Neg	ND
Fam 12	FB12	2945	21	A=1.7	β-thalassemia trait	Neg	Het	Neg	Neg
	M12	16/20		A ₂ =5.5		Neg	Het	Neg	ND
	F12	27/4		A ₂ =5		Neg	Het	Neg	ND

Raw data in fetal blood and parents at risk β-thalassemia/ Hb E disease

	No	PND Code	GA (Weeks)	HPLC	Diagnosis by HPLC	ARMS-PCR				
						Cd 17	Cd 41/42	Cd 71/72	IVS 1nt I G-T	Hb E
Fam 1	FB1	2054	19	A = 2.3 A ₂ = 3.6	Hb E trait	Neg	Neg	Neg	Neg	Het
	M1	784		A ₂ =6.5		Het	Neg	Neg	ND	ND
	F1	785		A ₂ =26.3		ND	ND	ND	ND	Het
Fam 2	FB2	2270	22	A = 7.9	Normal	Neg	Neg	Neg	Neg	Neg
	M2	236		A ₂ =22.7		ND	ND	ND	ND	Het
	F2	237		A ₂ =4.8		Het	Neg	Neg	ND	ND
Fam 3	FB3	2426	18	A = 0 A ₂ = 1.1	β-thalassemia / Hb E	Het	Neg	Neg	Neg	Het
	M3	25/23		A ₂ =26.3		ND	ND	ND	ND	Het
	F3	24		A ₂ =5.8		Het	Neg	Neg	ND	ND
Fam 4	FB4	2427	20	A = 1.5 A ₂ = 1.1	Hb E trait	Neg	Neg	Neg	Neg	Het
	M4	13/22		A ₂ =26.1		ND	ND	ND	ND	Het
	F4	14/22		A ₂ =6		Neg	Het	Neg	ND	ND
Fam 5	FB5	2430	20	A = 2.2	β- trait	Neg	Neg	Neg	Het	Neg
	M5	814		A ₂ =22.5		ND	ND	ND	ND	Het
	F5	815		A ₂ =4.2		Neg	Neg	Neg	Het	ND

Cotinue.

	No	PND Code	GA (Weeks)	HPLC	Diagnosis by HPLC	ARMS-PCR				
						Cd 17	Cd 41/42	Cd 71/72	IVS 1ntl G-T	Hb E
Fam 6	FB6	2485	18	A = 3.5	Normal	Neg	Neg	Neg	Neg	Neg
	M6	901		A ₂ =4.3		Neg	Neg	Neg	Het	ND
	F6	902		A ₂ =22.4		ND	ND	ND	ND	Het
Fam 7	FB7	2484	18	A = 3.0 A ₂ = 3.9	Hb E trait	Neg	Neg	Neg	Neg	Het
	M7	166		A ₂ =18.3		ND	ND	ND	ND	Het
	F7	167		A ₂ =5		Neg	Neg	Neg	Het	ND
Fam 8	FB8	2482	18	A = 2.7	Normal	Neg	Neg	Neg	Neg	Neg
	M8	185		A ₂ =5		Neg	Het	Neg	ND	ND
	F8	186		A ₂ =21.9		ND	ND	ND	ND	Het
Fam 9	FB9	2498	20	A = 6.1	Normal	Neg	Neg	Neg	Neg	Neg
	M9	19/29		A ₂ =5.7		Neg	Het	Neg	ND	ND
	F9	20/29		A ₂ =26.9		ND	ND	ND	ND	Het
Fam 10	FB10	2500	18	A = 2.8 A ₂ = 0.9	Hb E trait	Neg	Neg	Neg	Neg	Het
	M10	13/19		A ₂ =23.5		ND	ND	ND	ND	Het
	F10	14/19		A ₂ =6.3		Neg	Het	Neg	ND	ND
Fam 11	FB11	2837	20	A = 0 A ₂ = 0.5	β-thalassemia / Hb E	Neg	Neg	Neg	Neg	Het
	M11	35/12		A ₂ =8.1		Neg	Neg	Neg	Neg	ND
	F11	36/12		A ₂ =98.6		ND	ND	ND	ND	Homo
Fam 12	FB12	2610	18	A = 6.1	Normal	Neg	Neg	Neg	Neg	Neg
	M12	470		A ₂ =5.1		Het	Neg	Neg	ND	ND
	F12	471		A ₂ =25.4		ND	ND	ND	ND	Het
Fam 13	FB13	2675	18	A = 0 A ₂ = 1.2	β-thalassemia / Hb E	Het	Neg	Neg	ND	Het
	M13	29/29		A ₂ =25.5		ND	ND	ND	ND	Het
	F13	28/29		A ₂ =5.5		Het	Neg	Neg	ND	ND

Continue.

	No	PND Code	GA (Weeks)	HPLC	Diagnosis by HPLC	ARMS-PCR				
						Cd 17	Cd 41/42	Cd 71/72	IVS 1ntI G-T	Hb E
Fam 14	FB14	2642	18	A = 0.9	β -thalassemia trait	Neg	Het	Neg	ND	Neg
	M14	25		A ₂ =5.2		Neg	Het	Neg	ND	
	F14	23		A ₂ =26.3		ND	ND	ND	ND	Het

Raw data in CVS and parents at risk β -thalassemia/Hb E disease

	No	Diagnosis By HPLC	Diagnosis By nucleotide sequencing	ARMS-PCR				
				Cd 17	Cd 41/42	Cd 71/72	IVS 1ntI G-T	Hb E
Fam 1	CVS-1		Hb E trait	Neg	Neg	Neg	Neg	Het
	F1	β -trait		Het	Neg	Neg	ND	ND
	M1	Homo E		ND	ND	ND	ND	Homo
Fam 2	CVS-2		Heterozygous Cd 41/42	Neg	Het	Neg	Neg	Neg
	F2	E-trait		ND	ND	ND	ND	Het
	M2	β -trait		Neg	Het	Neg	ND	ND
Fam 3	CVS-3		Compound heterozygote Cd 17, Hb E	Het	Neg	Neg	ND	Het
	F3	β -trait		Het	Neg	Neg	ND	ND
	M3	E-trait		ND	ND	ND	ND	Het
Fam 4	CVS-4		Compound heterozygote Cd 17, Hb E	Het	Neg	Neg	ND	Het
	F4	β -trait		Het	Neg	Neg	ND	ND
	M4	Hb E trait		ND	ND	ND	ND	Het
Fam 5	CVS-5		Compound heterozygote Cd 17, Hb E	Het	Neg	Neg	ND	Het
	F5	β -trait		Het	Neg	Neg	ND	ND
	M5	Hb E trait		ND	ND	ND	ND	Het

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