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APPENDICES

APPENDIX I

Table XII : The primers for duplex selective PCR analysis

Code	Sense ^a	Location	Sequence (5'-3')
OU	+	1969-1990	5' – GGG GAA TTG GAG GTT TTA TCA A -- 3'
OL	-	2779-2796	5' – GTT CCT TCT GAT GCT TTT TGT CTG – 3'
IU	+	2321-2346	5' - GGA CAG CAC CAA ATG GAG GAA ATT AG –3
103WT ^b	-	2432-2461	5'-TCC CCC ACA TCT AGT ACT GTT ACT GAT TT -3'
103MT ^b	-	2432-2461	5'-TCC CCC ACA TCT AGT ACT GTT ACT GA TTG -3'
151WT	-	2574-2601	5'-AT ATT GCC GGT GAT CCT TTC CAT CC TTG -3'
151MT	-	2574-2601	5'-AT ATT GCC GGT GAT CCT TTC CAT CC AT -3'
181WT	-	2665-2684	5'-A TCC TAC ATA CGA GTC ATC CTT GTA TTG TT -3'
181MT	-	2665-2684	5'-A TCC TAC ATA CGA GTC ATC CTT GTA TTG TC -3'
215WT	-	2766-2788	5'-ATG ATG CTT TTT GTC TGG TGT <u>AGT</u> -3'
215MT	-	2766-2788	5'-ATG ATG CTT TTT GTC TGG TGT <u>AAA</u> -3'

^a + sense primer ; - antisense primer

^b the modified primers as described previously by Frater et al.⁽¹⁰⁶⁾

Base pairs comprising the codons of interest are in boldface type.

The sites of additional mismatches are in shading.

The nucleotide specific for Thai patients are in underline.

APPENDIX II

CHEMICAL AGENTS AND INSTRUMENTS

A. Chemical substances

Agarose (GIBCO; Grand Island, N.Y. USA)
Bromphenol blue (Sigma, MO, USA)
100 bp DNA ladder (Promega, USA)
dNTPs (Promega, USA)
Ethidium Bromide (Sigma, MO, USA)
Guanidium thiocyanate (Sigma, MO, USA)
Ficoll 400 (Sigma, MO, USA)
Isopropanol (Sigma, MO, USA)
RNAsin (Promega, USA)
MMLV-Reverse Transcriptase (Promega, USA)
Tag DNA Polymerase (Promega, USA)

B. Instrument

Agarose submarine gel apparatus
Automatic pipette (Gilson, Lyon, France)
Analytical Balance
Electrophoresis power supply (Biorad, CA, USA)
Glover, sterile
Incubator (Forma. Scientific, Ohio, USA)
Microcentrifuge (Eppendorf, USA)
Microcentrifuge tube
Mixer Vortex-Genic (Scientific industries, N.Y., USA)
Pipette tips
PCR machine Gene Amp PCR System 2400 (Perkin Elmer)
UV Trans-illuminator (ULTRA-LUM, Carson, California)

APPENDIX III

REAGENTS AND PREPARATIONS

1. 50X Tris-acetate buffer (TAE)

Tris-base	242.0 g
Glacial acetic acid	57.1 mL
0.5 M EDTA pH 8.0	100 mL

Adjust the volume to 1 liter with deionized distill water and sterilize by autoclaving at 121 °C for 15 min.

2. Working Electrophoresis buffer (1X TAE)

Stock 50X TAE	10 mL
Distill water	490 mL

3. 10 mg/mL Ethidium bromide (Et-Br) (Stock)

Ethidium bromide	1 g
DDW	100 mL

Stir on a magnetic stirrer for several hours to ensure that dye has dissolves. Wrap the container in aluminium foil or transfer to a dark bottle and stores at 4°C.

4. TAE for agarose gel preparation

1X TAE	1000 mL
stock 10 mg/mL Et-Br	5 µL

5. 1.5 % Agarose gel

Agarose ultrapure	1.5 g
1X TAE with Et-Br	100 mL

6. 6X loading buffer

Ficoll 400	20 %
Na ₂ EDTA, pH 8.0	0.1 M
Bromphenol Blue	0.25 %

BIOGRAPHY

Miss Chutitorn Ketloy was born on July 28, 1978 in Bangkok, Thailand. She received a Bachelors degree in Medical Technology from the Faculty of Allied Health Science, Chulalongkorn University in 1998. She enrolled at Chulalongkorn University in a graduate program for a master degree in Medical Science in 1999.

