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APPENDICES

APPENDIX A

BUFFERS AND REAGENT

1. Lysis Buffer I

Sucrose	109.54	g
1.0 M Tris – HCl (pH 7.5)	10	ml
1.0 M MgCl ₂	5	ml
Triton X – 100 (pure)	10	ml
Distilled water to	1,000	ml

Sterilize the solution by autoclaving and store in a refrigerator (at 4⁰C).

2. Lysis Buffer II

5.0 M NaCl	15	ml
0.5 M EDTA (pH 8.0)	48	ml
Distilled water to	1,000	ml

Sterilize the solution by autoclaving and store at room temperature.

3. 10% SDS solution

Sodium dodecyl sulfate	10	g
Distilled water to	100	ml

Mix the solution and store at room temperature.

4. 20 mg/ml Proteinase K

Proteinase K 2 mg

Distilled water to 1 ml

Mix the solution and store in a refrigerator (at -20°C).

5. 1.0 M Tris – HCl

Tris base 12.11 g

Dissolve in distilled water and adjusted pH to 7.5 with HCl

Distilled water to 100 ml

Sterilize the solution by autoclaving and store at room temperature.

6. 0.5 M EDTA (pH 8.0)

Disodium ethylenediamine tetraacetate. $2\text{H}_2\text{O}$ 186.6 g

Dissolve in distilled water and adjusted pH to 8.0 with NaOH

Distilled water to 1,000 ml

Sterilize the solution by autoclaving and store at room temperature.

7. 1.0 M MgCl_2 solution

Magnesium chloride. $6\text{H}_2\text{O}$ 20.33 g

Distilled water to 100 ml

Dispense the solution into aliquots and sterilize by autoclaving.

8. 5 M NaCl solution

Sodium chloride 29.25 g

Distilled water to 100 ml

Dispense the solution into aliquot and sterilize by autoclaving.

9. 10X Tris borate buffer (10X TBE buffer)

Tris – base 100 g

Boric acid 55 g

0.5 M EDTA (pH 8.0) 40 ml

Adjust volume to 1,000 ml with distilled water. The solution was mixed and store at room temperature.

10. 6X loading dye

Bromphenol blue 0.25 g

Xylene cyanol 0.25 g

Glycerol 50 ml

1M Tris (pH 8.0) 1 ml

Distilled water until 100 ml

Mixed and stored at 4°C

11. 7.5 M Ammonium acetate ($\text{CH}_3\text{COONH}_4$)

Ammonium acetate 57.81 g

Distilled water 80 ml

Adjust volume to 100 ml with distilled water and sterilize by autoclaving.

12. 25:24:1 (v/v) Phenol-chloroform-isoamyl alcohol

Phenol	25	volume
Chloroform	24	volume
Isoamyl alcohol	1	volume

Mix the reagent and store in a sterile bottle kept in a refrigerator.

13. 2% Agarose gel (w/v)

Agarose	1.6	g
1X TBE	80	ml

Dissolve by heating in microwave oven and occasional mix until no granules of agarose are visible.

14. Ethidium bromide

Ethidium bromide	10	mg
Distilled water	1	ml

Mix the solution and store at 4⁰C

APPENDIX B
SEQUENCE OF PRIMERS

Gene	Primer name	Location	Nucleotide sequence (5'>3')	Length (base pair)	Product size (base pair)	Digested products (base pair)	Tm (°C)
<i>CYP2E1</i>	CYP2E1-F	5'-flanking region	CCAGTCGAGTCTACATTGTCA	21	410	360, 50	47
	CYP2E1-R		TTCATTCTGTCTTCTAACTGG	21			43
<i>CR2</i>	CR2-II-F	Intron between exon1,2 and exon3	CTTTCTGTGCAGACCACGTT	20	1241	750, 491	47
	CR2-II-R		GATCTATGGTAGCCAGTTGG	20			47
<i>pIgR</i>	PIGRPVUII F	Intron3	TCAGCCAGGGTAAGGATCC	19	1392	1163, 129	48
	PIGRPVUII R		TGATGGTCACCGTTCTGCC	19			48

APPENDIX C
RETRITION NUCLEASE SITE

Gene	Restriction enzyme	Restriction site	Heterozygosity
<i>CR2</i>	<i>TaqI</i>	5' NNNNT*CGANNNN 3' 5' NNNNAGC*TNNNN 3'	42%
<i>pIgR</i>	<i>PvuII</i>	5' NNNNCAG*CTGNNNN 3' 5' NNNNGTC*GACNNNN 3'	45%
<i>CYP2E1</i>	<i>RsaI</i>	5' NNNNGT*ACNNNN 3' 5' NNNNCA*TGNNNN 3'	30%

BIOGRAPHY

Miss Narisorn Kongruttanachok was born on December 2, 1975 in Bangkok, Thailand. She received her Bachelor degree of Science (Medical Technology) in 1998 from Allied Health Science Faculty, Chulalongkorn University. She has enrolled Chulalongkorn University in graduate program for Master degree of Medical Science since 2001.

