APPENDIX

Appendix 1. List of the chemicals and materials used in the study.

Chemicals and Materials	Source
Absolute ethanol	J.T.Baker Inc., USA.
Acetic acid	J.T.Baker Inc., USA.
Ammonium sulfate	May&Baker Ltd., England
Acetylated Bovine Serum Albumin	Sigma Chemical Co. Ltd. USA.
Bis-acrylamide	Sigma Chemical Co. Ltd. USA.
Dimethyl sulfoxide (DMSO)	Sigma Chemical Co. Ltd. USA.
Ethidium bromide	Sigma Chemical Co. Ltd. USA.
Ethelenediamine tetraacetate (disodium salts)	Sigma Chemical Co. Ltd. USA.
Isopropanol	J.T.Baker Inc., USA.
Mineral oil	Promega Co. Ltd. USA.
Polyacrylamide	Sigma Chemical Co. Ltd. USA.
Proteinase-K	Sigma Chemical Co. Ltd. USA.
Seakem LE agarose	FMC Bioproducts. ME. USA.
Sodium acetate	Sigma Chemical Co. Ltd. USA.
Sodium chloride	Sigma Chemical Co. Ltd. USA.

Tris-hydroxy methyl aminomethane (Tris-base)	Sigma Chemical Co. Ltd. USA.
Triton x100	Sigma Chemical Co. Ltd.USA.
Wizard™ PCR PREP DNA purification system	Promega Co. Ltd. USA.

Cycle sequencing and sequencing chemicals.

Chain-termination cycle-sequencing ready reaction kit	Perkin Elmer Cetus, USA.
Popsix sequencing gel	Perkin Elmer Cetus, USA.
Template Suppression Reagent	Perkin Elmer Cetus, USA.

Deoxynucleotides triphosphates.

10 mM Deoxy-Adenine triphosphate	Promega Co. Ltd. USA.
10 mM Deoxy-Guanidine triphosphate	Promega Co. Ltd. USA.
10 mM Deoxy-Cytidine triphosphate	Promega Co. Ltd. USA.
10 mM Deoxy-Thymidine triphosphate	Promega Co. Ltd. USA.

DNA polymerases.

Taq DNA polymerase	Promega Co. Ltd. USA.
10x Thermophilic DNA polymerase buffer (supplied with *Taq* polymerase)	Promega Co. Ltd. USA.

Magnesium chloride (supplied with *Taq* polymerase)	Promega Co. Ltd. USA.
Vent$_R$® DNA polymerase	New England Biolabs, England.
10x Vent$_R$® DNA polymerase buffer (supplied with Vent$_R$® polymerase)	New England Biolabs, England.
Magnesium sulfate (supplied with Vent$_R$® polymerase)	New England Biolabs, England.

Oligonucleotide primers.

Forward primer "945"	Bio Service Unit, Mahidol University, Thailand.
Forward primer "946"	Bio Service Unit, Mahidol University, Thailand
Forward primer "947"	Bio Service Unit, Mahidol University, Thailand
Forward primer "948"	Bio Service Unit, Mahidol University, Thailand

Reverse primer "944"	Bio Service Unit, Mahidol University, Thailand
Reverse primer "2618"	Bio Service Unit, Mahidol University, Thailand

Photography accessary

Kodak developer (for black and white film)	Kodak Co. Ltd. USA.
Kodak developer (for cold-tone black and white paper)	Kodak Co. Ltd. USA.
Kodak fixer (for film and paper)	Kodak Co. Ltd. USA.
AGFAPAN (APX100) (film for black and white prints)	Agfa-Gevaert AG, Germany
Paper for black and white prints	Prince Co. Ltd. Thailand.

Restriction enzymes.

12 U/µl *Alw44* I	Promega Co. Ltd. USA.
10 mg/ml BSA (supplied with *Alw44* I)	Promega Co. Ltd. USA.
Reaction buffer"C" (supplied with *Alw44* I)	Promega Co. Ltd. USA.
20 U/µl *Stu* I	Promega Co. Ltd. USA.
10 mg/ml BSA (supplied with *Stu* I)	Promega Co. Ltd. USA.
Reaction buffer "B" (supplied with *Stu* I)	Promega Co. Ltd. USA.

Appendix 2. List of instruments used in the study

Instrument	Model	Source
Analytical balance	AC 100	Mettler Instrument A.G, Switzerland.
pH meter	-	Eutech Cybernetics, Singapore.
Ultra centrifuge		Japan
Refigerator (-20°C)		Sanyo, Thailand
Refigerator (-80°C)		Sanyo, Thailand
Spectrophotometer	Milton Roy, "1001 plus"	USA.
Water bath	Yamaha, type 1	Japan
Pipetteman (max. vol. 5 µl)		Scorax, USA.
Pipetteman (max. vol. 10 µl)		BIOHIT, USA.
Pipetteman (max. vol. 20 µl)		Pipetteman, USA.
Pipetteman (max. vol. 1000 µl)		Pipetteman, USA.
Pipetteman (max. vol. 5000 µl)		Pipetteman, USA.
Yellow tip for pipetteman (for 20-200 µl)		Treff-Switzerland
White tip for pipetteman (for 5-10 µl)		Sorrenson, USA.

Blue tip for pipetteman (for 1000 µl)		Treff-Switzerland
White tip for pipetteman (for 5000 µl)		Treff-Switzerland
Thermal cycler	"Touchdown"	Hybaid Co. Ltd. England
Long wavelength UV-transluminator		USA.
Camera	Nikon "Asahi"	Japan
ABI 310 Prism DNA sequencer	"310"	Perkin Elmer Cetus, USA.
Mini-Protean II Cell® electrophoresis apparatus		Bio-Rad Co. Ltd. USA.
Submarine agarose gel		Bio-Rad Co. Ltd. USA.
Power supply		Bio-Rad Co. Ltd. USA.
5 ml syringe barrel and plunger		Thailand

Appendix 3. Reagent preparationS

3.1. Tris-EDTA (TE) buffer pH 8.0

Tris-base	120.0 mg
EDTA	37.0 mg
Distilled water	e.q.

After being completely dissolved, the pH was adjusted to 8.0 by 1N HCl and then the volume was adjusted to 10 ml with distilled water. The solution was stored at -20°C.

3.2. 1M Tris buffer pH 8.4

Tris-base	1.21 g
Distilled water	e.q.

After being completely dissolved, the pH was adjusted to 8.4 by 1N HCl and then the volume was adjusted to 10 ml with distilled water. The solution was stored at -20°C.

3.3. 10 mM EDTA pH 8.4

EDTA	37.22 mg
Distilled water	e.q.

After being completely dissolved, the pH was adjusted to 8.4 by 1N HCl and then the volume was adjusted to 10 ml with distilled water. The solution was stored at -20°C.

3.4. 50x Tris-Acetic acid-EDTA (TAE) electrophoresis buffer (stock).

Tris-base	48.40 g
Glacial Acetic acid	11.42 ml
EDTA	3.72 g
Distilled water	e.q.

After being completely dissolved, the volume was adjusted to 200 ml with distilled water. The solution was stored at room temperature.

3.5. 10x *Taq* 2Δ buffer pH 8.4

1M Tris pH 8.4	450.0 µl
1 M ammonium sulfate	110.0 µl
2-mercaptoethanol	67.0 µl
10 mg/ml heated inactivated-	
Bovine Serum Albumin (BSA)	11.3 µl
Distilled water	317.7 µl

For the preparation of 1ml of 10x*Taq*2Δ buffer pH 8.4, 11.3 µl of stock 10 mg/ml Acetylated BSA was combined with 317.7 µl of

distilled-deionized water in a 0.5 ml PCR tube. The tube was briefly mixed and then incubated at 80°C for 20 min. The tube was left to cool down at room temperature and briefly centrifuged to collect evaporation from the tube wall. Each, stock solution was combined into 1.5 ml microfuge tube and then combined with the *Taq* 2Δ buffer. The solution was aliquate, 100 µl each, and be kept at -70°C for long time storage.

3.6. 1 mM each dNTPs stock solution

10 mM dATP	50 µl
10 mM dGTP	50 µl
10 mM dCTP	50 µl
10 mM dTTP	50 µl

Each portion of dATP, dGTP, dCTP and dTTP was combined to a 1.5 ml microfuge tube. The final volume was adjusted to 500 µl with distilled-deionized water. The adjustment of the pH of the solution to neutral was observed not necessary. The solution was stored at -20°C.

3.7. 10 mM each Primer stock solution.

Primer name	MW (crude delivered in 1 ml water)	OD	pmol/OD	stock concentration (mM)
944	7554.80	6.25	4167.00	26.04
945	5745.80	14.00	5263.00	73.68
946	5095.40	17.05	5882.00	100.29
947	4755.20	13.30	6250.00	83.13
948	5025.20	7.40	6250.00	46.25
2618	7796.00	7.62	4000.00	30.48

Each stock solution of primers was stored at -20°C. 10 mM of the working solution, 50 ml each, was prepared. The working solutions were stored at -20°C.

3.8. 3 mM Sodium acetate (NaOAc) pH 5.6

Sodium acetate	4.08 mg
Distilled-deionized water	e.q.

After being completely dissolved, the pH was adjusted to 5.6 by 1N HCl and then the final volume was adjusted to 10 ml with distilled water. The solution was stored at -20°C.

3.9 1.5 and 3 % agarose gel

gel percentage	agarose (mg)	50x TAE (ml)	distiled water (ml)
1.5	1.5	2.0	98.0
3.0	3.0	2.0	98.0

2.0 ml of 50x TAE buffer and 98 ml of distilled water were combined into a 250 ml autoclavable glass bottle with a screw-top lid. The glass bottle was continuously shaken on the plate-shaker machine. Then, the premeasured agarose powder was sprinkled into this bottle. Agarose was soaked in the buffer for 15 min before heating for reduce the tendency to form foam during heating.

3.10. 40% stock polyacrylamide solution.

Acrylamide	38.0 mg
Bis-acrylamide	2.0 mg
Distilled water	e.q.

Acrylamide and bis-acrylamide powder was first dissolved in 50 ml of distilled water in a 250 ml Erlenmyer flask. Stirring rod and hot plate machine were used for the continuously mixing of the acrylamide solution. The solution was warmed up on a hot plate to help redissolve

the solution. After being dissolved, the final volume was adjusted to 100 ml with distilled water and stored in a dark-bottle at 4°C.

3.11. 5% polyacrylamide gel.

40% stock polyacrylamide solution	750.0 µl
Distiled water	5.89 ml
10x TBE buffer	120.0 µl
TEMED	5.0 µl
10 mg/ml ammonium persulfate	36.0 µl

All stock solution, except ammonium persulfate, were combined in a 10 ml beaker and gently mixing. Ammonium persulfate was combined with the gel solution, and smoothly mixed just before pouring into a gel casting set.

VITA

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Educations :

March, 1985	Certificate of Mathayom III Suankularb College, Bangkok.
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Application :

Steger HF, Dhumtanom P and Sanguansermsri T (1994) : A simple 15-minute PCR sample preparation from whole blood. *Asia Pac J Mol Bio Biot* 2(4) : 368-369.