

APPENDIX

Appendix A

List of the chemicals and materials used in the study

Chemicals and Materials	Source
Absolute ethanol	Merck
Agarose	Sigma Chemical Co. Ltd., USA
Bovine Serum Albumin	Sigma Chemical Co. Ltd., USA
Boric acid	Sigma Chemical Co. Ltd., USA
Chelex-100 (iminodiacetic acid)	Sigma Chemical Co. Ltd., USA
Distilled water (sterile)	Maharaj Nakorn Chiang Mai Hospital Chiang Mai, Thailand
Ethylene diamine tetraacetate (disodium salts)	Sigma Chemical Co. Ltd., USA
Ethidium bromide	Sigma Chemical Co. Ltd., USA
Ficoll 400	Pharmacia Ltd., Sweden
Glycerol	Merck
Magnesium chloride	May & Baker Ltd., England
QIA quick PCR Purification kit	QIAGEN Ltd., Germany
Sodium hydroxide	Merck
Sodium acetate	Merck
Triton -X 100	Sigma Chemical Co. Ltd., USA
Tris-hydroxy methyamino methane	Sigma Chemical Co. Ltd., USA

Cycle sequencing and sequencing chemicals

Buffer for sequencing	Perkin Elmer Cetus, USA.
Chain-termination cycle sequencing ready reaction kit	Perkin Elmer Cetus, USA.
Popsix sequencing gel	Perkin Elmer Cetus, USA.
Template Suppression Reagent (TSR)	Perkin Elmer Cetus, USA.

Deoxynucleotides triphosphates

Powder of Deoxy-Adenosine triphosphate	Sigma Chemical Co. Ltd.,USA.
Powder of Deoxy-Cytidine triphosphate	Sigma Chemical Co. Ltd.,USA.
Powder of Deoxy-Guanidine triphosphate	Sigma Chemical Co. Ltd.,USA.
Powder of Deoxy-Thymidine triphosphate	Sigma Chemical Co. Ltd.,USA.

DNA polymerase

<i>Taq</i> DNA polymerase	Faculty of Associated Medical Science, Chiang Mai University, Chiang Mai, Thailand.
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Oligonucleotide primers

Forward primer CD7	Bioservice Unit, Faculty of Science, Mahidon University, Thailand.
Forward primer β 2A	Medical School, Hannover, Germany.
Forward primer FSA	Medical School, Hannover, Germany.
Forward primer EX3-S	MWG-Biotech GmbH, Germany.
Reverse primer β E1	Bioservice Unit, Faculty of Science, Mahidon University, Thailand.
Reverse primer FS41-42B	Medical School, Hannover, Germany.
Reverse primer EX3-A	MWG-Biotech GmbH, Germany.

Appendix B

List of instruments used in the Study

Instrument	Source
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	Sartorius, Germany.
Blue tip for pipetman (for 1000 μ l)	Treff-Switzerland .
Heating block	Eppendorf, Germany.
Electrophoresis apparatus	C.B.S. Scientific, CA.
Gel Doc 1000	Bio-Rad Co. Ltd., USA.
High speed refrigerated centrifuge	Italy.
Long wavelength UV	Supertonic Co. Ltd., NY.
Microcentrifuge tube (1.5 ml)	Treff-Switzerland.
Microcentrifuge	Italy.
PCR machine (system 2400)	Perkin Elmer Cetus, USA.
Power supply	C.B.S. Scientific , CA.
Spectrophotometer	USA.
Thin wall PCR tubes (0.2 ml)	Scientific Co. Ltd., CA.
White tip for pipetman (for 10 μ l)	Treff-Switzerland.
Yellow tip for pipetman (for 20-200 μ l)	Treff-Switzerland.

Appendix C

Reagent preparations

1. 3% agarose gels were prepared in 0.5X TBE.

2. 1 mM each of dNTPs

Stock solution

From dATP, dCTP, dGTP, dTTP powder, each of the dNTPs was dissolved in water and neutralized with NaOH, Tris-HCl (pH 7.2), and final concentrations 6 mM and 10 mM, respectively. The solution of 4 mM dATP, 4 mM dCTP, 4 mM GTP, 4 mM TTP were obtained and stored at -70°C . (Steger, unpublished)

Working solution of 1 mM concentrated dNTPs.

Containing:

4 mM dATP	250	μl
4 mM dCTP	250	μl
4 mM dGTP	250	μl
4 mM dTTP	250	μl

The solution was mixed and stored at -20°C .

3. Ethidium bromide solution

Containing:

10 mg/ml Ethidium bromide	7.0	μl
1X TBE	75.0	ml
Distilled water	75.0	ml

The solution was mixed and stored in a glass tray that was covered with aluminium foil for protection from light and stored at room temperature. It was made fresh every week.

4. 10 X loading buffer

Containing:

- 0.2% Xylene cyanol
- 0.3% Bromophenol blue
- 1.0% SDS
- 15% Ficoll (type 400)
- 50 mM Na₂EDTA

Distilled water was added to make 10 ml of solution and stored at room temperature.

5. Lysis buffer

Containing:

- 0.5 %Triton-X 100

The solution was stored at 4°C.

6. Primer stock solution dissolved in water.

Each stock solution of primers, 15 µM CD7, 15 µM βE1, 295 µM β2A, 70 µM FS41-42B, 87µM FSA, 100 µM EX3-S and 100 µM EX3-A were stored at -20°C. Working solution 5 µM and 3.2 µM were prepared in 50 µl aliquots. The working solutions were stored at -20°C.

7. 10X PCR buffer

Containing:

- 0.05% Tween 20
- 0.1 mg/ml BSA
- 1.5 mM MgCl₂
- 20 mM Tris-HCL, pH 8.4
- 50 mM KCl

After being completely dissolved, the volume was adjusted to 50 ml with distilled water and stored at -20°C.

8. 1X Tris–boric acid ethylenediamine tetraacetate (TBE) solution

Containing:

89 mM Tris-base

89 mM H₃BO₃

2 mM EDTA

After being completely dissolved, the volume was adjusted to 1,000 ml with distilled water. The solution was stored at room temperature.

9. 0.5X Tris–boric acid ethylenediamine tetraacetate (TBE) solution

Containing:

1X TBE 500 ml

Distilled water 500 ml

The solution was mixed and stored at 4°C.

10. 0.42 units/μl *Taq* DNA polymerase

Containing:

5 units/μl *Taq* DNA polymerase 2.5 μl

Glycerol 15.0 μl

10X PCR buffer 3.0 μl

Distilled water 9.5 μl

The solution was mixed and stored at -20°C.

11. 0.83 units/ μl *Taq* DNA polymerase

Containing:

5 units/ μl *Taq* DNA polymerase 5.0 μl

Glycerol 15.0 μl

10X PCR buffer 3.0 μl

Distilled water 7.0 μl

The solution was mixed and stored at -20°C.

VITA

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