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ศูนย์วิทยทรัพยากร  
จุฬาลงกรณ์มหาวิทยาลัย



# APPENDIX

ศูนย์วิทยทรัพยากร  
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## APPENDIX

### CHEMICAL AGENTS AND INSTRUMENTS

#### A. Chemical agents.

Absolute Ethanol (E. merck, Darmstadt., W. Germany)

Acetic acid (E. merck, Darmstadt., W. Germany)

Agarose (FMC Bioproducts, USA)

Bromphenol blue (Sigma, USA)

Diethyl Pyrocarbonate (Sigma, Mo., USA)

DNTP (Promega, WI., USA)

Ethylene diamine tetraacetic acid (Sigma, Mo., USA)

Ethidium bromide (Sigma, Mo., USA)

Sodium acetate (E. merck, Darmstadt., W. Germany)

#### B. Enzymes and molecular markers.

Taq DNA Polymerase (Promega, WI.,USA)

Rnasin Ribonuclease Inhibitor (Promega, WI.,USA)

M-MLV Reverse Transcriptase (Promega, WI.,USA)

100 bp molecular marker (Promega, WI.,USA)

#### C. Instruments.

Automatic pipette, P10/P20/P200/P1000 (Gilson, France)

DNA Thermal cycler 2400 (Perkin Elmer, Cetus USA)

Microcentrifuge (Eppendorf, USA)

Refrigerated Microcentrifuge (Eppendorf, USA)

Mixer Vortex-Genic (Scientific Industries., USA)

Laminar Air Flow (Faster, Italy)

Horizontal gel electrophoresis (C.B.S. Scientific., USA)

#### D. Laboratory supplies.

Glassware (Pyrex, USA)

Microcentrifuge tubes 1.5 ml (Costar, England)

Filter pipette tips for P10, P20, P200, P1000 (Treff Lab, Switzerland)

#### E. REAGENTS

##### 1. 0.01% DEPC-treated water.

Added 100 ul of DEPC into 1 litre of distilled water and left overnight,

Then autoclaved for 15 minutes at 15 lb/sq inch.

##### 2. Reagents for Reverse Transcriptase.

-5X RT buffer: 200 mM Tris-HCl pH 8.4, 500 mM KCl, 25 mM  $MgCl_2$

-25 mM  $MgCl_2$  (Promega, WI., USA)

-1.25 mM of each dNTP (dATP, dGTP, dCTP, dTTP) (Promega, WI., USA)

-50 pmol/ul Primer 2 in DEPC-treated water (BSU)

-40 units of Rnasin (Promega, WI., USA)

-100 units of M-MLV RT (Promega, WI., USA)

##### 3. Reagents for First PCR amplification.

-10X buffer: 500 mM KCl, 200mM Tris-HCl pH 8.4, 25 mM  $MgCl_2$

(Pharmacia Amersham)

-50 pmol/ul Primer 1 in DEPC-treated water (BSU)

-2.5 units of Taq DNA polymerase (Pharmacia Amersham)

##### 4. Reagents for nested PCR.

-10X buffer. 500 mM KCl, 200mM Tris-HCl pH 8.4, 25 mM  $MgCl_2$

(Pharmacia Amersham)

- 25 mM MgCl<sub>2</sub> (Promega, WI.,USA)
- 1.25 mM of each dNTP (dATP, dGTP, dCTP, dTTP) (Promega, WI.,USA)
- 50 pmol/ul Primer 3 in DEPC-treated water (BSU)
- 50 pmol/ul Primer 4 in DEPC-treated water (BSU)
- 2.5 units of Taq DNA polymerase (Pharmacia Amersham)

#### 5. Reagents for agarose gel electrophoresis.

- 2% agarose gel in 1X TAE buffer
- TAE buffer: 89 mM Tris-HCl pH 7.4, 89 mM acetic acid, 2.5 mM EDTA)
- loading dye solution
- 0.2% (v/v) ethidium bromide solution
- molecular size marker (100 bp, Promega)

#### F. Reagent of DNA Sequencing

ABI PRISM<sup>™</sup> Dye Terminator Cycle Sequencing Ready Reaction kit with  
Amplitaq<sup>®</sup> DNA Polymerase FS, (Perkin-Elmer, USA)

ศูนย์วิทยทรัพยากร  
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# BIOGRAPHY

<b>Name</b>	Miss Dararatsamee Poopukdee	<b>Sex</b>	Female
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