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APPENDICES

APPENDIX I

CHEMICAL AGENT AND INSTRUMENTS

A. Chemical substances

Agarose (GIBCO; Grand Island, N.Y. USA)

AmpliTaq Glod DNA polymerase (Perkin elmer, USA)

Bromphenol blue (Sigma, MO, USA)

Diethyl Pyrocarbonate ($C_6H_{10}O_5$) (Sigma, MO, USA)

100 bp DNA ladder (Promega, USA)

Ethanol (C_2H_5OH) (Merck, Germany)

Ethidium bromide (Sigma, MO, USA)

MMLV-Reverse Transcriptase (Promega, USA)

dNTPs (Promega, USA)

rNTPs (Promega, USA)

Phenol, Equilibrated (Merck, Germany)

2-Propanol ($CH_3CH(OH)CH_3$) (Merck, Germany)

QIAamp Spin Columns (QIAGEN Gmbh, Germany)

QIAamp Viral RNA Mini Columns (QIAGEN Gmbh, Germany)

QIAquick Spin Columns (QIAGEN Gmbh, Germany)

RNA Markers, 0.28-6.58 kb (Promega, USA)

RNasin Ribonuclease Inhibitor (Promega, USA)

*Sma*I restriction enzyme (Roche, Germany)

Sodium acetate (pH5.2) (Promega, USA)

Taq DNA polymerase (Promega, USA)

T7 RNA Polymerase (Promega, USA)

B. INSTRUMENTS

Real time PCR machine ABI 7700 PCR System (Perkin elmer, USA)

Agarose submarine gel apparatus

Automatic pipette (Gilson, Lyon, France)

Analytical balance

2-ml Collection tubes

Electrophoresis power supply (Biorad, CA, USA)

Microcentrifuge (Eppendorf, Germany)

-20 °C Freezer (sunyo, Japan)

-80 oC Freezer (Asheville, N.C., USA)

Glover, sterile

Incubator (Forma Scientific, Ohio, USA)

Microwave (Sharp, Japan)

Mixer-Vertex-Genic (Scienticfic industries, N.Y., USA)

pH meter (Orion, USA)

Pipette tip

PCR machine GeneAmp PCR System 9600 (Perkin elmer, USA)

Refrigerator (Sharp, Japan)

UV transilluminator (Bio-Rad, Canada)

APPENDIX II

REAGENTS AND PREPARATIONS

1) Reagents for plasmid purification

1.1) Luria-Bertani broth

Tryptone	10	g
Yeast extract	5	g
NaCl	10	g

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

1.2) Luria-Bertani agar plate

Tryptone	10	g
Yeast extract	5	g
NaCl	10	g
Agar	10	g

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

To pour plates, allow agar to cool about 50 °C was added ampicillin 100 mg/ml. After drying, store plates at 4 °C until used.

1.3) QIAGEN plasmid purification solution.

Add the provide RNase A solution to Buffer P1 before use. Use one vial of RNase A per bottle to Buffer P1, to give a final concentration of 100 μg/ml.

Check Buffer P2 for SDS precipitation due to low storage temperatures. If necessary, dissolve the SDS by warming to 37 °C.

Pre-chill Buffer P3 at 4 °C.

2) Reagents for viral RNA extraction.

2.1) QIAamp® Viral RNA solution.

Add 1 ml of Buffer AVL to one tube of lyophilized Carrier RNA. Dissolve Carrier RNA thoroughly. Transfer to the Buffer AVL bottle, and mix thorouhly before using Buffer AVL for the firat time.

Check Buffer AVL for precipitate, and if necessary incubated at 80 °C until the precipitate is dissolved (not more than 5 minutes)

3) Reagents for DNA agarose gel electrophoresis

3.1) 50X Tris-acetate buffer (TAE)

Tris-base	242	g
Glacial acetic acid	57.1	g
0.5 M EDTA pH 8.0	100	ml

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

3.2) Working Electrophoresis buffer (1X TAE)

Stock 50X TAE	10	ml
Distill water	490	ml

3.3) 10 mg/ml Ethidium bromide (Er-Br)(stock)

Ethidium bromide	1	g
Distill water	100	mL

Stir on a magnetic stirrer for several hours to ensure that dye has dissolved. Wrap the container in aluminum foil or trasfer to a dark bottle and store at 4 °C.

3.4) 2% Agarose gel

Agarose ultrapure	2	g
1X TAE with Et-Br	100	ml

3.5) 6X loading buffer

Ficoll 400	20%	
Na2EDTA, pH8.0	0.1M	
Bromphenol Blue	0.25%	

4) Reagent for RNA agarose gel electrophoresis

4.1) 1M EDTA

EDTA	7.44	g

Adjust pH to 8.0 with NaOH and adjust the volume to 10 milliliter with DNase-RNase free water and sterilized by autoclaving at 121 °C for 15 min

4.2) 5X MOPS buffer

MOPS	20.93	g
Sodium acetate	2.05	g
EDTA	0.5	M

Adjust pH to 7.0 with NaOH and adjust the volume to 500 milliliter with DNase-RNase free water and sterilized by autoclaving at 121 °C for 15 min

4.3) RNA sample buffer

Formamide	1	ml
37% formaldehyde	250	µl
5X MOPS buffer	200	µl

Sterilized by filtration and stored at -20 °C.

4.4) RNA loading buffer

50% glycerol		
EDTA	1	mM
Bromphenol Blue	0.04	g

Adjust the volume to 10 milliliter with DNase-RNase free water and sterilized by filtration and stored at – 20 °C.

4.5) 2% gel agarose

Agarose ultrapure	1	g
5X MOPS buffer	10	ml
DEPC-trated water	36	ml

Allow agar to cool about 50 °C was added 8.8 mL of 37% formaldehyde and 1 µl of 200 ng/ml Et-Br.

BIOGRAPHY

Miss Piyamat Jinnopat was born on May 25, 1978 in Trang, Thailand. She previously graduated with the Bachelors degree of Science (Microbiology), Faculty of Science, Prince of Songkhanakarin University, Songkha, Thailand in 2001.