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APPENDICES

APPENDIX I

Reagents, Materials and Instruments

A. Media and Reagents

Absolute ethanol	(Merck, Geramany)
Acetone	(Merck, Geramany)
Agarose (ultrapure)	(GIBCO BRL, U.S.A.)
*Bam*HI	(Invitrogen, U.S.A.)
dNTPs	(Invitrogen, U.S.A.)
Bovine Serum Albumin	(Sigma, U.S.A.)
Dimethyl sulfoxide (DMSO)	(Sigma, U.S.A.)
*Eco*RI	(Invitrogen, U.S.A.)
Ethylenediamine tetraacetic (EDTA)	(Amreso, U.S.A.)
Ethydium bromide	(Amreso, U.S.A.)
Fetal bovine serum	(sigma, U.S.A.)
Gum tragacanth	(sigma, U.S.A.)
HEPES	(GIBCO BRL, U.S.A.)
*Hin*dIII	(Invitrogen, U.S.A.)
*Kpn*I	(Invitrogen, U.S.A.)
L-glutamine	(sigma, U.S.A.)
Magnesium chloride ($MgCl_2$)	(Merck, Geramany)
Medium 199 (M199)	(GIBCO BRL, U.S.A.)
Penicilin G, Streptomycin	(GIBCO BRL, U.S.A.)
Phenol (equilibrated)	(USB, U.S.A.)
Phenol : Chlorofrom : Isoamy (ultrapure)	(USB, U.S.A.)
Taq DNA polymerase (with $MgCl_2$ and PCR buffer)	(GIBCO BRL, U.S.A.)
Tris-base	(sigma, U.S.A.)
Trypsin	(sigma, U.S.A.)

B. Materials

Microcentrifuge tube	(TRS, U.S.A.)
Tissue culture flask	(Nunclon, Denmark)
Tissue culture plate	(Nunclon, Denmark)
Cell scarper	(Costar®, mexico)

C. Instruments

Autoclave (model-S90N)	(Tomyseiko, Japan)
Chemi doc	(Bio-Rad, U.S.A.)
DNA thermocycle system	(Hybaid, U.S.A)
Incubator type 80	(Memmert, Geramany)
Microcentrifuge	(Fotodyne, U.S.A.)
Power Supply (Model 1000/500)	(Bio-Rad, U.S.A.)
Refrigerator	(Toshiba, Japan)
Spectrophotometer (SmartSpect ™ 3000)	(Bio-Rad, U.S.A.)
Electrophoresis chamber	(CBS, California)

APPENDIX II

Reagents Preparation

Reagents and Media for Cell Culture

1. 2X Medium 199

M 199 with Earle's salts, with L-glutamine, without $NaHCO_3$	9.9 g
Sterilized deionized distilled water	500 ml
Filtration and stored at 4 °C	

2. 1 M HEPES

HEPES (N-2-hydroxyethyl-piperaine-N'-2-ethan sulfonic acid)	23.83 g
Deionized distilled water	100 ml
Sterilized by autoclaving 121 °C 15 minutes	

3. 10% $NaHCO_3$

$NaH\ CO_3$	10 g
Deionized distilled water	100 ml
Sterilized by autoclaving 121 °C 15 minutes	

4. L-glutamine

L-glutamine	2.922 g
Sterilized deionized distilled water	100 ml
Fitration and stored at -20 °C	

5. 10% M 199 medium (GM)

2x M 199 with Earle's salts, with L-glutamine without $NaHCO_3$	50 ml
1 M HEPES	1 ml
Penicilin/Streptomycin antibiotic (10^5unit/ml)	0.1 ml
10% $NaHCO_3$ adjusted to pH 7.4	1 ml
L-glutamine	1 ml
Fetal bovine serum	10 ml
Deionized distilled water	37 ml

6. 2% M 199 medium (MM)

2x M 199 with Earle's salts, with L-glutamine without $NaHCO_3$	50 ml
1 M HEPES	1 ml
Penicilin/Streptomycin antibiotic (10^5unit/ml)	0.1 ml
10% NaH CO_3 adjusted to pH 7.4	1 ml
L-glutamine	1 ml
Fetal bovine serum	2 ml
Sterilized deionized distilled water	45 ml

7. 10 X PBS (Phosphate-buffer saline)

NaCl	40 g
KCl	1 g
$NaHPO_4$	5.75 g
KH_2PO_4	1 g
Deionized distilled water to	1000 ml
Sterilized by autoclaving 121 °C 15 minutes	

8. 1X PBS

10 X stock PBS	100 ml
Sterilized deionized distilled water	900 ml

9. 10X Trypsin

Trypsin	0.5 g
EDTA	0.2 g
NaCl	9 g
Deionized distilled water to	100 ml

Sterilized by filtration and stored at -20 °C

10. 1X Trypsin

10X stock trypsin	10 ml
Sterilized deionized distilled water	90 ml

stored at 4 °C

11. Plaque overlay medium

Solution A

10X M199 with Earl's salts, with L-glutamine without $NaHCO_3$	20 ml
Fetal bovine serum	20 ml
1M HEPES	2 ml
Penicilin/Streptomycin antibiotic (10^5unit/ml)	0.2 ml
L-glutamine	2 ml
10% $NaHCO_3$ adjusted to pH 7.4	3 ml
Sterilized deionized distilled water	42 ml

Solution B

Gum tragacanth	1.6 g
Deionized distilled water	100 ml

Sterilized by autoclaving at 121 °C 15 minutes

Solution A and B were mixed at the ratio 1:1 before use.

Reagents for Preparation of Viral DNAs

1. Lysing solution

Triton X-100	0.25 %
EDTA	10 ml
Tris, pH 7.9	10 ml

2. 5 M NaCl

NaCl	292.20 g
Deionized distilled water to	1,000 ml

Sterilized by autoclaving at 121 °C 15 minutes

3. RNase A (10mg/ml)

RNase A	10 mg/ml
Tris, pH 7.5	10 mM
NaCl	15 mM

Heated to 100 °C for 15 minutes and stored at 4°C

4. Proteinase K (20mg/ml)

Proteinase K	20 mg/ml
Tris, pH 8.0	0.01 M
EDTA	0.005 M
SDS	0.5 %

Stored at 4°C

5. 3 M Sodium acetate

Sodium acetate $3H_2O$	40.8 g
Deionized distilled water to	100 ml

Adjusted to pH 5.2 with glacial acid

6. TE buffer (Tris/EDTA)

Tris, pH 7.4	10 mM
EDTA, pH 8.0	1 mM

Reagents for Gel Electrophoresis

1. Tris-borate buffer (TBE)

Tris –base	54 g
Boric acid	27.5 g
5 M EDTA, pH 8.0	20 ml
Sterilized by autoclaving	

2. Loading dye

Bromphenol blue	0.25 g
Xylene cyanol FR	0.25 g
Glycerol	30 ml
Deionized distilled water	69.5 ml

BIOGRAPHY

Miss. Sutida Visaprom was born on February 25, 1978 in Ubonratchatani, Thailand. She previously graduated with the Bachelor degree of Science in Microbiology from the Faculty of Science, Ubonratchatani University in 2000.