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APPENDICES

APPENDIX I

REAGENTS AND INSTRUMENTS

A. REAGENT

Absolute ethanol	(Merck,U.S.A)
Agarose (ultrapure)	(Biorad, U.S.A)
Boric acid	(Amresco, U.S.A.)
ECL detection reagent	(Amresco, U.S.A.)
EDAC	(Sigma, U.S.A.)
EDTA	(Amresco, U.S.A)
Ethidium bromide	(Amresco, U.S.A)
Methanol	(Merck, U.S.A)
Mineral oil	(Sigma, U.S.A)
N-acetyl-*L*-cysteine	(Sigma, U.S.A)
NaCl	(Sigma, U.S.A)
$NaHCO_3$	(Sigma, U.S.A.)
Na_2HPO_4	(Sigma, U.S.A)
Sodium citrate	(Sigma, U.S.A)
Sosium dodecyl sulphate	(Amresco, U.S.A)
Sodium hydroxide	(Merck, Germany)
Streptavidin-peroxidase conjugate	(Amresco, U.S.A)
KH_2PO_4	(Sigma, U.S.A)
Tris (ultrapure)	(Amresco, U.S.A)
Tris hydrochloride	(Amresco, U.S.A)

B. MATERIAL

Biodyne C membrane	(Amresco, U.S.A.)
Hyperfilm ECL	(Amresco, U.S.A.)
X-ray film	(Kodak, Japan)

APPENDIX I (CONTINUE)

C. INSTRUMENTS

Hybaid OmniGene thermal cycler	(Hybaid, England)
Camera Gel Doc™ MZL	(Bio-RAD, USA)
Dot botter	(BBL, U.S.A.)
Water bath	(Memmert, U.S.A)
Incubator	(Forma Scientific, U.S.A)
Microcentrifuge	(Eppendorf, U.S.A)
Perkin Elmer GeneAmp PCR system 9600	(Perkin Elmer, U.S.A)
ABI Prism™ 310 Automate sequencer	(Perkin Elmer, U.S.A)
Spectrophotometer	(BIORAD, U.S.A.)

APPENDIX II

MEDIA FOR CULTURE

1. Ogawa media

Mineral salt solution

Potassium dihydrogen phosphate anhydrous (KH_2PO_4)	3.0	g
Sodium glutamate	3.0	g
Distilled water	300	ml
Glycerine	18	ml
2%Malachite green solution	18	ml
Homogenised whole eggs	600ml (12-16 eggs)	

Autoclaving mineral salt solution at 121°C for 15 minutes to sterilise. Cool to room temperature. The following ingredients are aseptically pooled in a large, sterile flask and mixed well: glycerine, 2% malachite green solution, homogenised whole eggs. The medium is mixed well and distributed in 6-8 ml volumes in sterile 20x150 mm screw-capped test tubes. Place the bottles in a slanted position in the inspissator and coagulate the medium for 45 minutes at 80-85 °C. Cool and store at 4 °C until used.

APPENDIX III

REAGENTS AND PREPARATIONS

1. 0.5 M Ethylene diamine tetraacetic acid (EDTA), pH 8.0

Dissodium ethylene diamine tetraacetate.$2H_2O$	186.1	g
DDW	800.0	ml

Adjust pH to 8.0

Adjust volume to 1,000 ml

Sterililize by autoclaving

2. 1 M Tris-HCl, pH 8.0

Tris hydrochloride	157.64	g
DDW	800.0	ml

Adjust pH to 8.0

Adjust volume to 1,000 ml

Sterililize by autoclaving

3. 5x Tris-borate buffer (TBE)

Tris base	54	g
Boric acid	27.5	g
0.5 M EDTA (pH 8.0)	20	ml

Adjust volume to 1 liter with distilled water. The solution was mixed and sterilized by autoclaving at 121°C for 15 min.

4. 10 x TE buffer

Tris	12.11	g
0.5 M EDTA	20	ml

Adjust to pH 8.0 by adding conc. HCl, adjust volume to 1,000 ml. Sterilize by autoclaving

5. 20xSSPE

Na_2HPO_4	28.4	g
NaCl	210.24	g
EDTA	7.4	g

Adjust to pH 7.4, Adjust volume to 1,000 ml. Sterilize by autoclaving.

Store at room temperature for no longer than one years.

6. 10% SDS

SDS	10	g
DDW	100	ml

Dissolave by heating at 65°C for 20 min. Do not autoclaving

7. 0.5 M $NaHCO_3$, pH 8.4

$NaHCO_3$	10.5	g
DDW	250	ml

Adjuat to pH 8.4, adfuat volume to 250 ml with DDW

Store at room temperature for no longer than one years.

8. 16% (w/v) EDAC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide)

EDAC	1.6	g
DDW	10	ml

Prepare fresh before use.

9. 2XSSPE

20XSSPE	20	ml
DDW	180	ml

Prepare fresh before use.

10. 20 mM EDTA

0.5 M EDTA (pH 8.0)	4	ml
DDW	96	ml

Prepare fresh before use.

11. 2XSSPE/0.1% SDS

20XSSPE	20	ml
10% SDS	2	ml
DDW	178	ml

Prepare fresh before use.

12. 2XSSPE/0.5% SDS

20XSSPE	20	ml
10% SDS	10	ML
DDW	170	ml

Prepare fresh before use.

13. ECL detection reagent

ECL detection reagent 1	5	ml
ECL detection reagent 2	5	ml

Store at 4°C for no longer than six months.

14. 0.1 M NaOH

NaOH	0.4	g
DDW	100	ml

Prepare fresh before use.

15. 0.5 M Na-citrate

Na-citrate	147	g
DDW	1,000	ml

Sterilize by autoclaving.

16. 5 M NaOH

NaOH	200	g
DDW	1,000	ml

Sterilize by autoclaving.

17. Alkaline wash solution (0.05 M Na-citrate and 0.5 M NaOH)

0.5 M Na-citrate	5	ml
5 M NaOH	5	ml
DDW	40	ml

Sterilize by autoclaving.

18. 0.5 M Tris-HCl, pH 8.0

Tris hydrochloride	78.82	g
DDW	800.0	ml

Adjust pH to 8.0, adjust volume to 1,000 ml

Sterililize by autoclaving

APPENDIX III (CONTINUE)

REAGENT FOR AGAROSE GEL ELECTROPHIRESIS

1. 10 mg/ml Ethidium bromide

Ethidium bromide	1	g
DDW	100	g

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

2. 2% Agarose gel

Agarose (ultrapure)	0.4	g
1 x TBE	20.0	ml
10 mg/ml Ethidium bromide	1.0	µl

Dissolve by heating in microwave oven and occasional mix unit no granules of agarose are visible.

BIOGRAPHY

Miss Sanjira Juntarapornchai was born on July 31, 1980 in Bangkok, Thailand. She graduated with bachelor degree of science in Microbiology from the Faculty of Science at Chulalongkorn University in 2002.