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APPENDIX I

REAGENTS, MATERIALS AND INSTRUMENTS

A. REAGENTS

Absolute ethanol	(Merck, Germany)
Acrylamide/bisacrylamide	(Biorad, U.S.A.)
Agarose (ultrapure)	(Amresco, U.S.A.)
Ammonium persulfate	(Biorad, U.S.A.)
Ampicillin sodium salt	(Amresco, U.S.A.)
Boric acid	(Merck, Germany)
Coomassie brilliant blue R 250	(Biorad, U.S.A.)
Dextrose	(Difco, U.S.A.)
Developer	(Kodak, Japan)
Ethidium bromide	(Amresco, U.S.A.)
EDTA	(Amresco, U.S.A.)
Fixer	(Kodak, Japan)
Glacial acetic acid	(Merck, Germany)
Glycine	(Sigma, U.S.A.)
Lambda DNA/ <i>Hind</i> III	(BRL, U.S.A.)
Methanol	(Merck, U.S.A.)
Phenol	(BRL, U.S.A.)
Restriction enzymes	(Gibco, U.S.A.)
Sodium dodecyl sulfate	(Amresco, U.S.A.)
Sodium hydroxide	(Merck, Germany)
N,N,N,N-tetramethylethylenediamine (TEMED)	(Biorad, U.S.A.)

Tris (ultrapure)	(Amresco, U.S.A.)
Tryptone	(Lab m, U.S.A.)
Urea	(Promega, U.S.A.)
X-gal	(Amresco, U.S.A.)

B. MATERIALS

X-ray	(Fuji, Japan)
Chromatography paper no.3	(Whatmann, England)
Pre-cut sheet of porous cellophane	(Flexel, U.S.A.)

C. INSTRUMENTS

Sequencing gel model SA-60	(BRL, U.S.A.)
Horizon 58 horizontal gel electrophoresis system	(BRL, U.S.A.)
Slab gel system model DASG-250	(C.B.S., Scientific, U.S.A.)
Spectrophotometric GeneSys	(Milton Roy, U.S.A.)
Gel dryer	(Biorad, U.S.A.)

APPENDIX II

REAGENTS AND PREPARATIONS

1. 0.5 M EDTA, pH 8.0

Disodium ethylene diamine tetraacetate.2H ₂ O	186.1 g
DDW	800.0 ml
Adjust pH to 8.0	
Adjust volume to 1000 ml	

2. 3M Sodium acetate, pH 5.0

Sodium acetate.3H ₂ O	408.1 g
DDW	800.0 ml
Adjust pH to 5.0 with glacial acetic acid	
Adjust volume to 1000 ml	

3. 1 M Tris-Cl, pH 8.0

Tris (ultrapure)	121.1 g
DDW	800.0 ml
Adjust to pH 8.0 by adding conc. HCl	42.0 ml
Sterilize by autoclaving	

4. 50X Tris-acetate buffer

Tris (ultrapure)	242.0 g
Glacial acetic acid	57.1 ml
0.5 M EDTA pH 8.0	100.0 ml
Adjust volume to 1000 ml with DDW	
Sterilize by autoclaving	

5. TE buffer

1 M Tris-Cl, pH 8.0	0.5 ml
0.5 M EDTA, pH 8.0	0.2 ml
DDW	9.3 ml

MEDIA AND PREPARATIONS

1. LB (Luria-Bertani) broth

Tryptone	10.0 g
Yeast extract	5.0 g
NaCl	5.0 g
1 N NaOH	1.0 ml

Adjust volume to 1000 ml

Sterilize by autoclaving

2. LB+ Ampicillin medium

LB broth 100.0 ml

Adding Ampicillin to final concentration of 50 $\mu\text{g/ml}$

at 45 °C after autoclaving

3. Tryptone-glucose broth

Beef extract 3.0 g

Tryptone 5.0 g

Dextrose 10.0 g

DW 1000.0 ml

Sterilize by autoclaving

4. Tryptone-glucose agar

Beef extract 3.0 g

Tryptone 5.0 g

Dextrose 10.0 g

Agar 15.0 g

DW 1000.0 ml

Sterilize by autoclaving

5. X-gal (20 mg/ml)

X-gal 0.02 g

Dimethylformamide 1.0 ml

APPENDIX III

I. REAGENTS AND GEL PREPARATION FOR SEQUENCING GEL

1. 6% polyacrylamide gel (60 ml)

urea	25.2	g
10x Tris-borate buffer	6.0	ml
40% acrylamide/2% bisacrylamide	9.0	ml
H ₂ O	26.0	ml
TEMED	40.0	μl
10% Ammonium persulfate	400.0	μl

2. 10x Tris-borate buffer

Tris	108.0	g
Boric acid	55.0	g
0.5 M EDTA	40.0	ml

Adjust volume to 1000 ml with H₂O

Sterilize by autoclaving

3. 10% Ammonium persulfate

Ammonium persulfate	1	g
H ₂ O	10	ml

freshly preparation before used

II. REAGENTS AND GEL PREPARATION FOR SDS-PAGE

1. 12% separating gel (20 ml)

40% acrylamide/2% bisacrylamide	6.00	ml
4x Tris-Cl/SDS pH 8.8	5.00	ml
H ₂ O	8.92	ml
10% Ammonium persulfate	68	μl
TEMED	12	μl
freshly preparation before used		

2. 4% stacking gel (8 ml)

40% acrylamide/2% bisacrylamide	0.78	ml
4x Tris-Cl/SDS pH 6.8	2.00	ml
H ₂ O	5.17	ml
10% Ammonium persulfate	40	μl
TEMED	8	μl
freshly preparation before used		

3. 4X Tris-Cl/SDS, pH 6.8

Tris	6.01	g
SDS	0.4	g
H ₂ O	40	ml

Adjust to pH 6.8 with 1 N HCl

Add H₂O to 100 ml

4. 4x Tris-Cl/SDS, pH 8.8

Tris	18.17	g
SDS	0.40	g
H ₂ O	60	ml

Adjust to pH 8.8 with 1 N HCl

Add H₂O to 100 ml

5. 1x SDS electrophoresis buffer

Tris	3.0	g
Glycine	14.5	g
SDS	1.0	g

Adjust to pH 8.3

Add H₂O to 1000 ml.

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BIOGRAPHY

Miss Saowanee Kwanlertjit was born on September 14, 1967 in Bangkok, Thailand. She graduated with the Bachelor of Science (Medical Technology) degree from Faculty of Medicine , Chulalongkorn University in 1990. She works as a scientist at Department of Microbiology, Faculty of Medicine, Chulalongkorn University.



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