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APPENDICES

APPENDIX A

100x BG-11 without Fe, Phosphate, Carbonate (1 liter)

149.60 g	$NaNO_3$
7.49 g	$MgSO_4.7H_2O$
3.60 g	$CaCl_2.2\ H_2O$
0.89 g	Sodium citrate
1.12 ml	0.25 M NaEDTA, pH 8.0
100 ml	Trace Mineral

Trace Mineral (1 liter)

2.86 g	H_3BO_3
1.81 g	$MnCl_2.4H_2O$
0.222 g	$ZnSO_4.7\ H_2O$
0.39 g	$Na_2MoO_4.2\ H_2O$
0.079 g	$CuSO_4.5\ H_2O$
0.494 g	$Co(NO_3)_2.6\ H_2O$

1000x Ferric ammonium citrate

600 mg per 100 ml H_2O

1000x Na_2CO_3

2 g Na_2CO_3 per 100 ml H_2O

1000x K_2HPO_4

3.05 g K_2HPO_4 per 100 ml H_2O

BG-11 liquid media (1 L)

10 ml 100xBG-11 without Fe, phosphate, carbonate

1 ml 1000xferric ammonium citrate

1 ml 1000xNa_2CO_3

1 ml 1000xK_2HPO_4

BG-11 solid agar plates (1 L)

10 ml 100xBG-11 without Fe, phosphate, carbonate

1 ml 1000xferric ammonium citrate

1 ml 1000xNa_2CO_3

1 ml 1000xK_2HPO_4

10 ml 1M TES/NaOH buffer, pH 8.2

3 g Na-thiosulfate

15 g Bactoagar

Autoclve 121°C, 15 lb/in^2 both liquid and agar media for 20 min.

APPENDIX B

LB media (1 liter)

10 g Bactotryptone

5 g Yeast extract

10 g NaCl

Dissolve all component with 1,000 ml deionized water. Autoclave at 121 °C, 15 lb/in^2 for 15 min. For media containing agar add bactoagar 15 g per liter.

APPENDIX C

Reagent for heat-shock transformation

ΨB media (500 ml)

2.5 g Yeast extract

10 g Bactotryptone

0.38 g KCl

Adjust the pH to 7.6 with KOH then autoclave. After autoclaving, cool down and add sterile 1 M $MgSO_4$ 17 ml.

TfBI solution (500 ml)

1.47 g Potassium acetate

4.95 g $MnCl_2$

6.05 g RbCl

0.74 g $CaCl_2$

75 ml Glycerol

Adjust the pH to 5.8 with 0.2 M acetic acid and autoclave. Store at 4°C.

TFBII (100 ml)

10 ml 100 mM MOPS, pH 7.0

1.10 g $CaCl_2$

0.12 g RbCl

15 ml Glycerol

Autoclave and store at 4°C in the wrap bottle in tinfoil.

APPENDIX D

Reagent for alkaline lysis miniprep and sequencing miniprep

Solution 1 (100 ml)

5.0 ml 1.0 M Glucose

2.5 ml 1.0 M Tris-HCl, pH 8.0

2.0 ml 0.5 M EDTA, pH 8.0

Autoclave and store at 4°C.

Solution 2 (25 ml)

0.5 ml 10 M NaOH

1.25 ml 20% SDS

Solution 3 (500 ml)

147 g potassium acetate

57.5 ml glacial acetic acid

Autoclave and store at 4°C.

TE buffer (500 ml)

5 ml 1 M Tris-HCl, pH 8.0

1 ml 0.5 M EDTA

Autoclave and store at room temperature.

APPENDIX E

Reagent for DNA agarose gel electrophoresis

5x TBE (1 liter)

54.0 g Trizma base

27.5 g Boric acid, anhydrous

20.0 ml 0.5 M EDTA, pH 8.0.

Loading dye (20 ml)

0.05 g bromophenol blue

0.05 g xylene cyanol FF

6.0 ml Glycerol

Make up to 20 ml with milli-Q water.

APPENDIX F

PCR amplification mixture

Taq Polymerase 10x Buffer

100 mM ris-HCl, pH 8.3

500 mM KCl

0.1%(w/v) Gelatin

Autoclave and Store in 100 µl aliquots at -20°C.

PCR 2x Reaction Mix

100 µl Taq Polymerase 10x Buffer

0.4 mM each dNTP

5 µg primers

3 mM $MgCl_2$

Make up to 500 µl with sterile milli-Q water.

PCR

In a 0.6 ml microfuge tube add:

25 µl PCR 2x Reaction Mix

0.5 µl Platinum Taq

10 ng - 1 µg genomic DNA

Make up to 50 µl with sterile milli-Q water.

APPENDIX G

Reagent for Southern Blot

20x SSC (1 liter)

175.3 g NaCl

88.2 g Na_3 citrate.$2H_2O$

800 ml milli-Q water

Adjust the pH to 7.0 with 1 M HCl and make to 1 liter with milli-Q water.

100x Denhardts solution (50 ml)

1 g Ficoll 400

1 g Polyvinylpyrolidone

1 g BSA (Fraction V)

Make up to 50 ml with milli-Q water and mix. Filter-sterile, aliquot and store at -20°C.

Denhardts Pre-hyb Solution

5x Denhardts solution

1% SDS

5x SSC

50% Formamide

APPENDIX H

Reagents for making probe of Southern Blots

Solution O

1.25 M Tris-HCl, pH 8.0

0.125 M $MgCl_2$

Make up in 20 ml milli-Q water. Autoclaved and stored at -20°C.

Solution A

5 µl dGTP, 5 µl dATP, 5 µl dTTP

18 µl 14.4 M 2-mercaptoethonol

1 ml Solution O

Stored at -20°C.

Solution B

2M HEPES-NaOH, pH 6.6

Autoclaved and stored at 4°C.

Solution C

Random primer

Stop solution

20 mM Tris-HCl, pH 7.8

20 mM NaCl

2 mM EDTA

0.25% (w/v) SDS

Store at -20°C.

BSA Solution

10 mg/ml BSA

Store at -20°C.

OLB Solution

2 µl Solution A

5 µl Solution B

3 µl Solution C

APPENDIX I

Reagent for RNA gel electrophoresis

DEPC-water

1 ml DEPC

1 liter milli-Q water

Stirred for a couple of hours. Incubated overnight at 37°C. Autoclaved to inactivate the DEPC.

10x MOPS buffer (1 liter)

0.2 M MOPS

0.05 M Sodium acetate, pH 5.0

0.01 M EDTA, pH 8.0

Adjust to pH 7.0 with NaOH. Wrap in tin foil and kept at room temperature.

2x RNA loading Buffer (1 ml)

500 µl formamide

160 µl formaldehyde

100 µl 10x MOPS

100 µl Glycerol

105 µl sterile DEPC-water

25 µl 10 mg/ml bromophenolblue

10 µl 10 mg/ml ethidium bromide

APPENDIX J

Reagents for pTZ 19U mutagenesis

High Salt Buffer

300 mM NaCl

100 mM Tris-HCl, pH 8.0

1 mM EDTA

Autoclave and Store at -20°C.

DTT Solution

1 M DTT in 0.01 M Sodium acetate, pH 5.2

Dilute to 0.1 M with 0.01 M Sodium acetate, pH 5.2

Store at -20°C.

Neutralized ATP

0.1 M ATP, pH 7.0 (adjust pH with 0.1 M NaOH)

Make up to 1 ml with water and dilute to 1 mM ATP

Store at -20°C.

10x Annealing Buffer

200 mM Tris-HCl, pH 7.4

20 mM $MgCl_2$

500 mM NaCl

Store at -20°C.

10x Synthesis Buffer

100 mM Tris-HCl, pH 7.9

5 mM each dNTP

10 mM ATP

50 mM $MgCl_2$

15 mM DTT

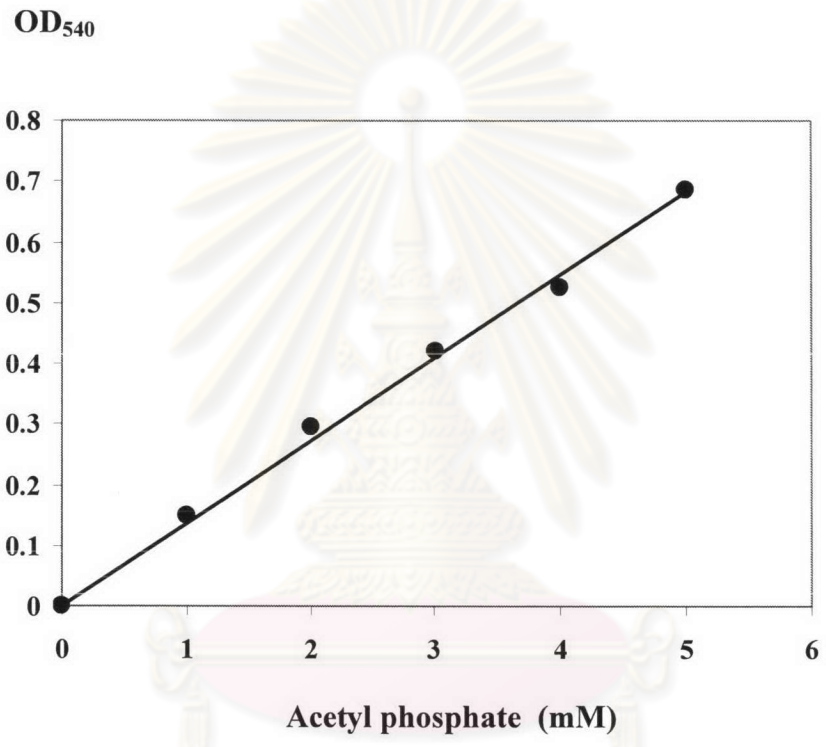
TE Stop Buffer

10 mM EDTA

10 mM Tris-HCl, pH 8.0

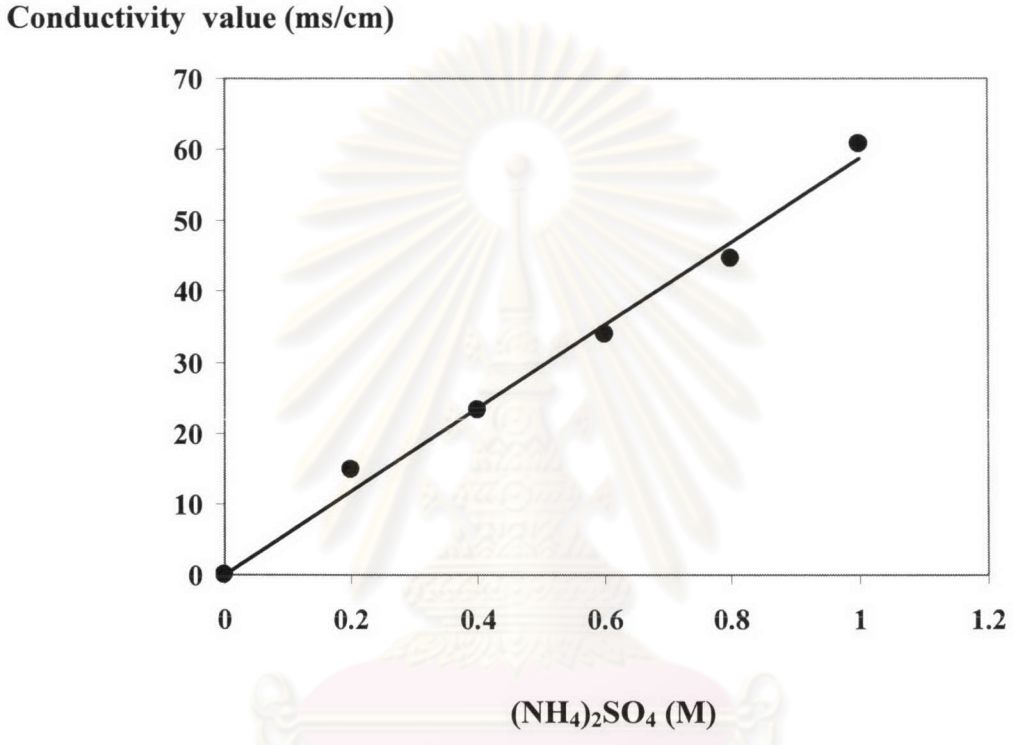
APPENDIX K

Standard curve of acetyl phosphate



APPENDIX L

Standard curve of $(NH_4)_2SO_4$



APPENDIX M

Bradford stock solution

100 ml 95%ethanol

200 ml 88%phosphoric acid

350 mg Serva Blue G

Bradford working solution

425 ml distilled water

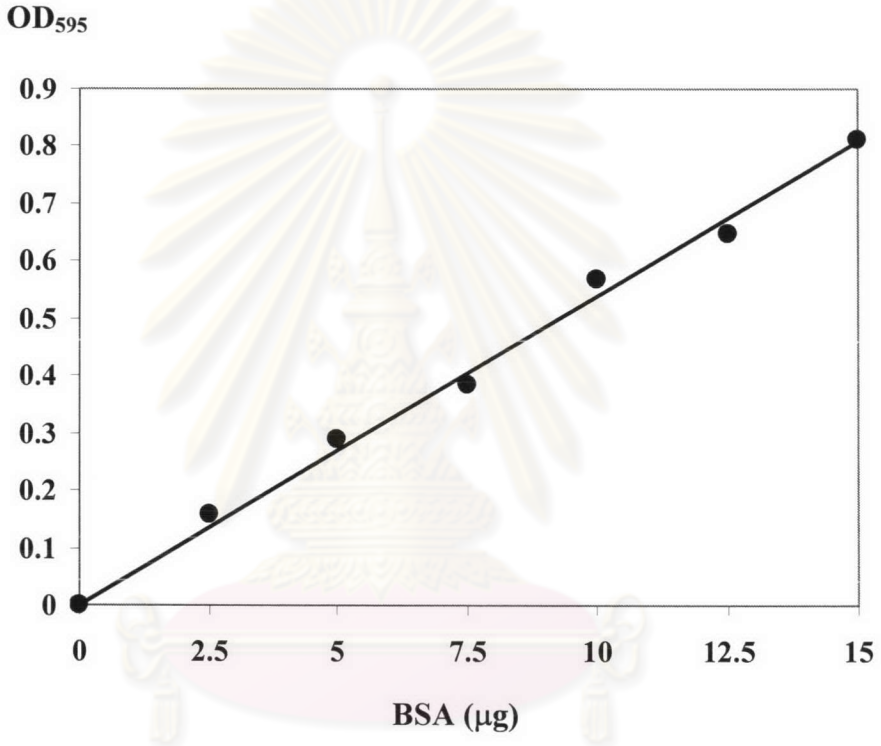
15 ml 95%ethanol

30 ml 88%phosphoric acid

30 ml Bradford stock solution

APPENDIX N

Standard curve for protein determination by Bradford's method



APPENDIX O

Preparation for polyacrylamide gel electrophoresis

Stock reagents

30% Acrylamide, 0.8% bis-acrylamide 100 ml

acrylamide	29.2 mg
N,N'-methylene-bis-acrylamide	0.8 g

1.5 M Tris-HCl, pH 8.8

Tris(hydroxymethyl)-aminomethane	18.17 g

Adjust pH to 8.8 with 1 M HCl and adjust volume to 100 ml with distilled water.

2 M Tris-HCl, pH 8.8

Tris(hydroxymethyl)-aminomethane	24.2 g

Adjust pH to 8.8 with 1 M HCl and adjust volume to 100 ml with distilled water.

0.5 M Tris-HCl, pH 6.8

Tris(hydroxymethyl)-aminomethane	6.06 g

Adjust pH to 6.8 with 1 M HCl and adjust volume to 100 ml with distilled water.

1 M Tris-HCl, pH 6.8

Tris(hydroxymethyl)-aminomethane	12.1 g

Adjust pH to 6.8 with 1 M HCl and adjust volume to 100 ml with distilled water.

Solution B (SDS-PAGE)

2 M Tris-HCl; pH 8.8	75 ml
10% SDS	4 ml
distilled water	21 ml

Solution C (SDS-PAGE)

1 M Tris-HCl; pH 8.8	50 ml
10% SDS	4 ml
distilled water	46 ml

SDS-PAGE

10% separating gel

30% acrylamide solution	3.33 ml
solution B	2.5 ml
distilled water	5.0 ml
10% $(NH_4)_2S_2O_8$	50 µl
TEMED	10 µl

5.0% stacking gel

30% acrylamide solution	0.67 ml
solution C	1.0 ml
distilled water	2.3 ml
10% $(NH_4)_2S_2O_8$	30 µl
TEMED	5 µl

Sample buffer

1 M Tris-HCl; pH 6.8	0.6 ml
50% Glycerol	5.0 ml
10% SDS	2.0 ml
2-mercaptoethanol	0.5 ml
1% bromophenol blue	1.0 ml
distilled water	0.9 ml

One part of sample buffer is added to four parts of sample. The mixture is heated 5 minutes in boiling water before loading to the gel.

Electrophoresis buffer (1 liter)

Tris(hydroxymethyl)-aminomethane	3.0 g
Glycine	14.4 g
SDS	1.0 g

Adjust volume to 1 liter with distilled water.

BIOGRAPHY

Miss Waraporn Juntarajumnong was born on March 28, 1978 in Bangkok, Thailand. She graduated with a Bachelor of Science degree in Biochemistry from Chulalongkorn University in 2000. She has further studied for the Master and Doctor of Philosophy degree in Biochemistry Department, Chulalongkorn university since 2000.