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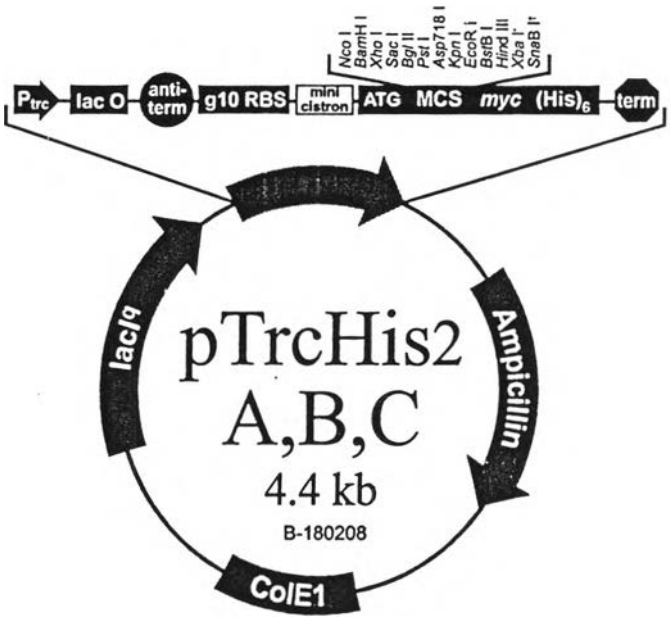
APPENDICES

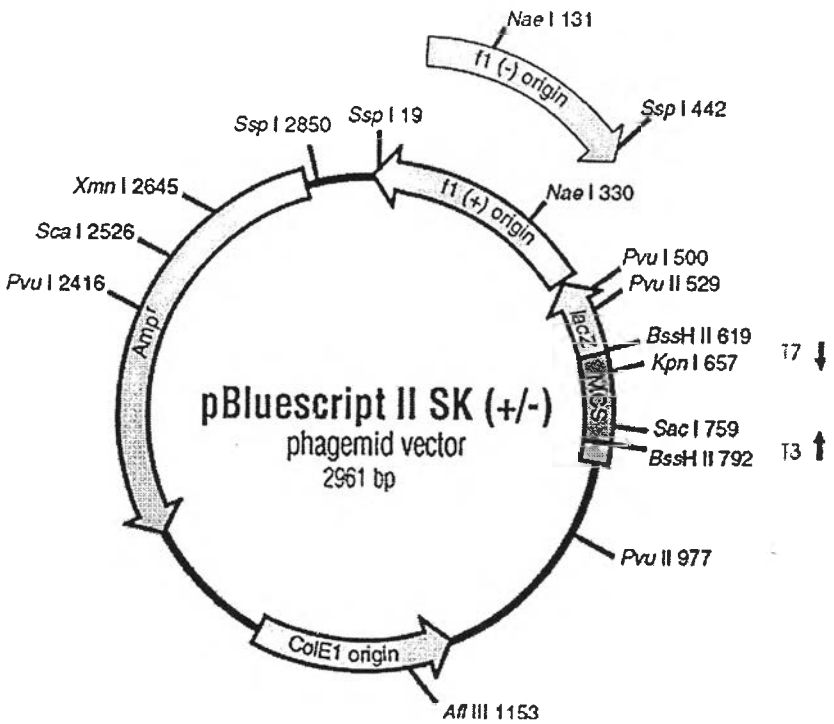
APPENDIX 1

pTrcHis2C



APPENDIX 2

pBluescript II SK+



APPENDIX 3

Transformation, Chang-Miller Method

1. Preparation of competent cells

A single colony of *E.coli* DH5α was inoculated to 2 ml of LB medium and inoculation at 37°C overnight with vigorous shaking. This culture was reinoculated to fresh LB medium and incubated 37°C with vigorous shaking for 3-4 hours until the OD620 reach 0.4-0.6 . The culture was standed on ice for 10 min and centrifuged at 4000 rpm for 10 min at 4°C. Cell pellet was resuspended in 0.05 volume of TSB-DMSO free medium and stand on ice for 10 min. This cell suspension was dispensed in 100 µl aliquots into 1.5 ml microcentrifuge tubes and stored at –70°C.

2. Transformation

One hundred microlitres of competent cells was thawed on ice prior to the addition of 1-3 µl of plasmid DNA or ligation mixture. The transformation mixture was flicked 2-3 times and stand on ice 10 min. Subsequently, the mixture was heated to 42°C for 90 second further on ice 5 min. The mixture was diluted with 918 µl of 1xTSB-DMSO-Glucose medium (855 µl 1xTSB, 45 µl DMSO and 18 µl 1M Glucose) and gently shaking at 37°C for 60 minutes. Cell suspension was spread on selection medium as desired.

1XTSB (DMSO free)

1 g	Bacto tryptone
0.5 g	Yeast extract
0.5 g	NaCl
10 g	PEG4000
1 ml	1 M $MgSO_4$
1 ml	1 M $MgCl_2$

Add all compositions with 80 ml distilled water and the pH to 6.1 with conc. HCl (approximately 20 µl). Then, add distilled water up to 100 ml and autoclave at 121 °C, 15 lb/in^2 for 15 minutes.

APPENDIX 4

Alkaline lysis method

A single colony of *E.coli* harboring recombinant plasmid was grown in 1.5 ml of LB solution containing 50 µl/ml ampicillin at 37°C for overnight with shaking. The cells were harvested by centrifugation at 4000 rpm for 10 minutes at 4°C and suspened in 100 µl of solution I (50 mM glucose, 25 mM Tris-Cl pH 8.0 and 10 mM EDTA) by vigorous vortexing. After 5 minutes incubation at room temperature, the cells were lysed by the addition of 200 µl of freshly prepared solution II (0.2 N NaOH and 1% SDS), mixed by gently inversion and incubated on ice for 5 minutes. The cells lysate was neutralized by gently mixing with 150 µl of 3 M sodium acetate pH 4.8 followed by 5 minutes incubation on ice. The mixture was centrifuged at 15,000 rpm for 5 min at 4°C. The clear lysate was collected, extracted once with phenol/chloroform/isoamylalcohol (25: 24: 1). Subsequently, the plasmid was precipitated by adding 2 volumes of ice-cold absolute ethanol, mixed by inversion several times before incubated at -20°C for 10 minutes and then centrifuged for 10 minutes at 15,000 rpm at 4°C. The plasmid was washed with 70% ethanol and recollected by centrifugation for 3 minutes. Finally, the air-dried pellet was dissolved in 20 µl TE buffer and stored at –20 °C.

APPENDIX 5

Agarose gel electrophoresis for DNA

To measure the size and the amount of DNA in the sample, 0.8 – 1.5 % agarose gel (consist of 0.5 mg/ml ethidium bromide) in 1xTBE buffer (89 mM Tris-Cl, 89 mM boric acid and 2.5 mM EDTA pH 8.3) consisting 0.5 mg/ml ethidium bromide was used. The DNA sample was mixed with 1/5 volume of loading dye (0.25% bromphenol blue, 0.25% xylene cyanolFF and 30% glycerol in water) before loading into the well of gel which submerged in the 1xTBE buffer in an electrophoretic chamber. An appropriate amount of λ/*Hind* III or λ/*Eco*RV was also load to the gel to serve as a DNA marker. Generally, the gel was run at 100 volts until bromphenol blue migrated to the other egde. The DNA band was visualized under UV light and photograph. The concentration and molecular weight of DNAs sample were estimated by comparing with the intensity and relative mobility of λ/*Hind* III or λ/*Eco*RV. The standard DNA bands λ/*Hind* III is 23.1, 9.4, 6.6, 4.4, 2.3, 2.0 and 0.5 kb, respectively.And for the standard DNA bands λ/*Eco*RV is 5.7, 5.3, 4.6, 3.8, 2.8, 2.6, 1.9, 1.6, 1.4, 0.7, 0.5, and 0.2 kb, respectively.

For the detection of RNA, 1.2 % agarose gel in MOPS buffer was prepared (0.7 g Seakem GTC agarose, 3 ml 20xMOPs and 45 ml milli-Q water). Agarose gel in MOPs buffer was autoclaved. After cooled down to 50-60°C, 3 ml of 37% formaldehyde solution, milli-Q water up to 60 ml and ethidium bromide (final concentration 0.5 mg/ml) was added in gel. The gel was mixed gently and poured into gel former. The gel will set and become untransparent about 30 min. The electrophoresis buffer for RNA was 1xMOPs containing 0.5 mg/ml ethidium bromide. The other step was done according to the protocol for DNA electrophoresis.

APPENDIX 6

RNA extraction buffer

Final concentration per 1 litre

200 mM Tris –Cl pH 9.0

100 mM NaCl

10 mM EDTA

0.5% SDS

14 mM β-mercaptoethanol

Dissolve all compositions with distilled water except SDS and β-mercaptoethanol. Autoclave at 121 ° C, 15 lb/in^2 for 15 minutes. After autoclaving, cool down and add SDS and β-mercaptoethanol.

APPENDIX 7

TCDS buffer

Composition per 1 litre

10 mM Tris –Cl pH 7.5

140 mM Choline Chloride

250 mM Sucrose

50 mM DTT

Dissolve all compositions with distilled water and keep at 4°C

APPENDIX 8

Buffer for western blotting

PBS buffer (Phosphate-buffer-saline)

Final concentration per 1 litre

10 mM sodium phosphate pH 7.4

150 mM NaCl

Blocking buffer

5% (w/v) skim milk and 0.01% Tween20 in PBS buffer

Blotting transfer buffer

Final concentration per 1 litre

39 mM glycine

48 mM Tris-base

0.037% SDS

20% methanol

APPENDIX 9

Detection reagent for western blotting

18 ml 150 mM Barbital pH 9.6

2 ml 0.1% NTB (Nitro Blue Tetrazolium)

80 µl 1 M $MgCl_2$

200 µl 0.5% BCIP (5-bromo-4-chloro-3-indolyl phosphate)

Detection reagent for western bloting should be freshly prepared and used within 30 minutes. When the bands are of the desired intensity, wash the nitrocellulose membrane with deionized water 2-3 times and take photograph.

APPENDIX 10

Preparation for polyacrylamide gel electrophoresis

1) Stock reagents

30% Acrylamide, 0.8% bis-acrylamide, 100ml

acrylamide	29.2 g
N, N′-methylene-bis-acrylamide	0.8 g

Adjusted volume to 100 ml with distilled water

1.5 M Tris-HCl pH 8.8

Tris (hydroxymethyl)-aminomethane	18.17 g

Adjusted pH to 8.8 with 1M HCl and adjusted volume to 100 ml with distilled water

2 M Tris-HCl pH 8.8

Tris (hydroxymethyl)-aminomethane	24.2 g

Adjusted pH to 8.8 with 1M HCl and adjusted volume to 100 ml with distilled water

0.5 M Tris-HCl pH 6.8

Tris (hydroxymethyl)-aminomethane	6.06 g

Adjusted pH to 6.8 with 1 M HCl and adjusted volume to 100 ml with distilled water

1 M Tris-HCl pH 6.8

Tris (hydroxymethyl)-aminomethane	12.1 g

Adjusted pH to 6.8 with 1M HCl and adjusted 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 6.8	50 ml
10% SDS	4 ml
distilled water	46 ml

2. 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 litre

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

Adjust volume to 1 litre with distilled water

(pH should be approximately 8.3).

APPENDIX 11

LB medium

Composition per 1 liter

10 g	Bacto tryptone
5 g	Yeast extract
10 g	NaCl

Dissolve all compositions with 800 ml deionized water, adjust the pH to 7.0 with 6M NaOH. Adjust volume of solution to 1 litre with deionized water. Autoclave at 121 ^{o} C, 15 lb/in^{2} for 15 minutes. For media containing agar add bactoagar 15 g per litre.

APPENDIX 12

LBK medium

Composition per 1 liter

10 g	Bacto tryptone
5 g	Yeast extract
10 g	KCl

Dissolve all compositions with 800 ml distilled water, adjust to final pH 7.0 with 6M KOH and add distilled water up to 1000 ml. Autoclave at 121 ° C, 15 lb/in^2 for 15 minutes. For media containing agar add bactoagar 15 g per litre.

For LBK + 0.2 M NaCl medium, NaCl is added to the final concentration 0.2 M after adjusted pH with KOH.

For LBK + 4 mM LiCl medium, LiCl is added to the final concentration 4 mM after adjusted pH with KOH.

APPENDIX 13

TrisE medium

Compositions per 1 litre

0.12 M Tris –Cl

0.07 M NaCl

0.02 M KCl

0.02 M NH_4Cl

$3x10^{-3}$ M Na_2SO_4

$1x10^{-3}$ M $MgCl_2.6H_2O$

$3x10^{-3}$ M $CaCl_2.2H_2O$

$3x10^{-3}$ M $ZnCl_4.2H_2O$

0.05% Bactopeptone

0.6 % Glycerol

Dissolve all compositions with distilled water and adjust the pH to 7.5 or 8.0 with 1M HCl. Autoclave at 121 ° C, 15 lb/in^2 for 15 minutes. After autoclaving, cool down and add $CaCl_2$ at final concentration of 100 mM

APPENDIX 14

Dragendorff's reagent

Stock solution

Solution A

Bismuth nitrate	17	g
Tartaric acid	200	g

Adjust volume to 800 ml with distilled water

Solution B

Potassium iodide	160	g

Adjust volume to 400 ml with distilled water

Mix solution A and B

For use, 100 g tartaric acid is dissolved in 50 ml of the mixture (solution A and B) and 250 ml water

APPENDIX 15

L-homocysteine preparation

L-homocysteine was freshly prepared from L-homocysteine thiolactone, the solid powder was dissolved in 1 M NaOH at final concentration 100 mM and incubated at room temperature 1 min. After that the alkali solution was adjusted pH with Tris-Cl and HCl to obtain final pH 8.3

APPENDIX 16

^{14}C-glycinebetaine preparation

^{14}C-glycinebetaine was prepared by the reaction of choline oxidase from *Alcaligenes* sp. (Ikuta et al., 1977). The radioactive substrate ^{14}C-choline (55 μCi/μ mol) was converted to ^{14}C-betaine aldehyde by adding choline oxidase and incubated 25°C for 4 h. The intermediate product, ^{14}C-betaine aldehyde was converted to ^{14}C-glycinebetaine by adding NaOH (final concentration 0.17 M) and H_2O_2 (final concentration 10%) and incubated overnight. The product, ^{14}C-glycinebetaine, was separated by ion exchange chromatography (Dowex 50W, 50x4-200, hydroxyl form) and eluted by 2 M NH_3. The solution was lyophilized and checked for the purity by autoradiography.

BIOGRAPHY

Miss Rungaroon Waditee was born on June 11, 1971 in Bangkok, Thailand. She graduated with a Bechelor of Science degree in Microbiology and Master of Science in Industrial Microbiology from Chulalongkorn University in 1992 and 1995 respectively. She has further studied for the Doctor of Philosophy (Ph. D.) degree in Biochemistry Department, Chulalongkorn University since 1998.