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APPENDICES

APPENDIX

1. **Chemicals for preparation the media**

1.1 Luria-Bertani broth (LB) total volume 100 ml.

Peptone	1.0	gm.
NaCl	1.0	gm.
Yeast extract	0.5	gm.

Dissolve in 100 ml. of deionized water. Adjust the pH of the solution to 7.0 using NaOH (sodium hydroxide). Autoclave at 121°C for 20 min to sterilized the broth.

The broth can be stored sealed at room temperature.

1.2 LB agar total volume 100 ml.

Peptone	1.0	gm.
NaCl	1.0	gm.
Yeast extract	0.5	gm.
Agar	1.5	gm.

Dissolve in 100 ml. of deionized water except agar. Adjust the pH of the solution to 7.0 using NaOH (sodium hydroxide). Add agar and autoclave at 121°C for 20 min to sterilize the broth.

1.3 SOB solution total volume 100 ml.

Yeast extract	0.5	gm.
Tryptone	2	gm.
1 mM NaCl	1	ml.
1 M KCl	0.25	ml.
1 mM $MgCl_2$	1	ml.
1 mM $MgSO_4$	1	ml.

Dissolve in 100 ml. of deionized water. Adjust the pH of the solution to 7.0 using NaOH (sodium hydroxide) or HCl (hydrochloric). Autoclave at 121°C for 20 min to sterilized.

1.4 SOC medium total volume 100 ml.

Yeast extract	0.5	gm.
Bacto tryptone	2.0	gm
NaCl	0.06	ml.
KCl	0.02	ml.

Dissolve in 98 ml. of deionized water. Autoclave at 121°C for 20 min to sterilized. Add 1.0 ml. of 1 M $MgSO_4$ and 1.0 ml. of 2M glucose. Sterilize by filtration with 0.2 µm filter.

2. Chemicals for preparation the competent cell

2.1 TB solution total volume 100 ml.

PIPES	0.3	gm.
$CaCl_2$	0.7	gm.
KCl	1.86	gm.

Dissolve in deionized water and adjust to pH 6.7 with NaOH or HCl and then add 1.09 gm. of $MnCl_2$. Adjust to final volume 100 ml. Sterilize by filtration with 0.2 µm filter and store at 4°C.

2.2 Dimethyl sulfoxide (DMSO) store at -20°C

3. Chemicals for cloning

3.1 50 µg/ml of ampicillin

Dissolve 0.5 mg of ampicillin in deionized water. Adjust to final volume 10 ml. Sterilize by filtration with 0.2 µm filter and store at -20°C.

Add 50 µg/ml. of ampicillin into LB broth.

3.2 Bromo-4-chloro-3-indolyl-ß-D-galactoside (X-Gal solution) concentration 20 mg/ml.

Dissolve 20 mg. of bromo-4-chloro-3-indolyl-ß-D-galactoside in 1 ml. of dimethylformamide (DMF). Store at -20°C.

3.3 Isopropyl thio-ß-D-galactoside (IPTG solution) concentration 24 mg/ml.

Dissolve 120 mg. of isopropyl thio-ß-D- galactoside in 5 ml. deionized water. Sterilize by filtration with 0.2 µm filter and store at -20°C.

4. DNA plasmid extraction kit (QIA Spin Miniprep Kit); QIAGEN®

Solution of the DNA plasmid extraction consists:

4.1 Lysis solution

4.2 Wash buffer

4.3 Elution buffer

4.4 Fast plasmid Spin column assembly

5. Chemicals for plasmid DNA extraction

5.1 Solutions for DNA detection using size of DNA in agarose gel electrophoresis

10x TAE buffer: volume 1 liter

Tris-base	48.44	gm.
$CH_3CooNa_3H_2O$	16.4	gm.
Na_2EDTA	7.44	gm.
Glacial acetic acid	17	ml.

Dissolve in deionized water and adjust to pH 7.7 with glacial acetic acid. Adjust to final volume 1 liter and autoclave at 121°C for 20 min.

5.2 Loading buffer solution

Bromophenol blue	0.01	gm.
Tris-HCl (pH 6.8)	1.25	ml.
Glycerol	5	ml.

Dissolve bromophenol blue and tris-HCl and then adjust to final volume 5 ml. with deionized water. Add 5 ml. of glycerol. Store at 4°C.

5.3 50 and 100 of DNA standard marker: Invitrogen®

6. **Chemicals for Polymerase Chain Reaction (PCR): Invitrogen®**

The Chemicals for PCR consists:

6.1 10x PCR buffer

6.2 2mM dNTP

6.3 25mM $MgCl_2$

6.4 *Taq* DNA polymerase

7. **Chemicals for *Wolbachia* extraction**

Chemicals for *Wolbachia* extraction is 0.5%gelatin+5%fetal bovine serum in PBS pH 7.4

Gelatin	0.025	gm.
Fetal bovine serum	250	ul

Dissolve 0.025 gm. of gelatin in 250 ul fetal bovine serum.

8. **Chemicals for DNA extraction**

8.1 Extraction buffer

8.1.1. 0.1M NaCl

1M	=	58.44 g/L
0.1M	=	5.844 g/L
	=	0.5844 g/100 ml

8.1.2 0.2M Sucrose

1M	=	342.3 g/L
0.1 M	=	34.23 g/L
0.2 M	=	68.46 g/L
	=	6.846 g/100 ml

8.1.3 0.1M Tris-HCl

1 M	=	121.14 g/L
0.1 M	=	12.114 g /L
	=	1.2114 g/100 ml

8.1.4 0.05M EDTA

1 M = 372.24 g/L

0.1 M = 37.224 g/L

0.05 M = 18.612 g/L

= 1.8612 g/100 ml

Dissolve in deionized water and adjust to pH 9.1 with NaOH. Adjust to final volume 100 ml and then add 0.5% of SDS (0.5 gm/100 ml.)

8.2 8M KAC

1M = 98.15 g/L

8M = 785.2 g/L

= 78.52 g/100 ml

= 39.26 g/50 ml

Dissolve 39.26 g in 5o ml deionized water.

8.3 0.1x SSC

8.3.1 15 mM NaCl

0.015 M NaCl

1 M = 58.44 g/L

0.015 M= 0.8766 g/L

= 0.08766 g/100 ml

8.3.2 1.5 mM Sodium Citrate

0.0015 M Sodium Citrate

1 M = 294.10 g/L

0.0015 M= 0.44115 g/L

= 0.044115 g/100 ml

Dissolve 0.04383 g NaCl and 0.0220575 g Sodium Citrate in 100 ml deionized water.

8.4 100% ethanol

9. **Chemicals for DNA ligation: Invitrogen®**

Chemicals for DNA ligation consists:

T4 DNA ligase

T4 DNA ligase buffer

Deionized water

BIOGRAPHY

Lt. Srettapong Thimaharn was born on December 26, 1970 in Nongkhai, Thailand. He received his Bachelor degree of science (Medical Technology) in 1994 from the Department of Medical Technology, Faculty of Medical Technology, Rangsit University, Bangkok, Thailand. He has enrolled in graduate program for master degree of Medical Science at Chulalongkorn University since 2006.