

APPENDIX

List of chemical and reagents used in this study

Name of chemicals	Company
1 kb plus DNA ladder	Fermentas Life Sciences, USA
Acetic acid	Lab scan, USA
Acrylamide	Sigma-Aldrich, USA
Acetonitrile	Sigma-Aldrich, USA
Agarose powder	USB, USA
Ammonium persulfate	Sigma-Aldrich, USA
Ammonium bicarbonate	Sigma-Aldrich, USA
Ampicillin	TP Drug laboratory
Arginine	Sigma-Aldrich, USA
Adenosine triphosphate(ATP)	Fermentas Life Sciences, USA
Adenine	Sigma-Aldrich, USA
Bovine serum albumin	Sigma-Aldrich, USA
Bromphenol blue	USB, USA
Calcium chloride(CaCl_2)	Merck, Germany
Chloroform	Merck, Germany
Coomassie blue	Sigma-Aldrich, USA
dNTPs	Fermentas Life Sciences, USA
D-glucose	Merck, Germany



Dithiothreitol(DTT)	Merck, Germany
Ethylenediamineetetraacetic acid(EDTA)	Fluka, USA
Ethanol	Scharlau, South Australia
Ethidium bromide	Sigma-Aldrich, USA
Glycerol	Sigma-Aldrich, USA
Gentamicin	M&H manufacturing
Giemsa solution	Merck, Germany
Guanine	Sigma-Aldrich, USA
HEPES	Sigma-Aldrich, USA
Hydrochloric acid	Merck, Germany
Isopropanol	Merck, Germany
Isopropyl β -D-1-thiogalactopyranoside(IPTG)	Merck, Germany
Kanamycin	M&H manufacturing
Leucine	Sigma-Aldrich, USA
Lysine	Sigma-Aldrich, USA
Magnesium chloride(MgCl ₂)	Sigma-Aldrich, USA
Magnesium sulfate(MgSO ₄)	Sigma-Aldrich, USA
Methanol	Merck, Germany
Methionine	Sigma-Aldrich, USA
Sodium bicarbonate(NaHCO ₃)	Sigma-Aldrich, USA
Sodium acetate(NaOAc)	Sigma-Aldrich, USA

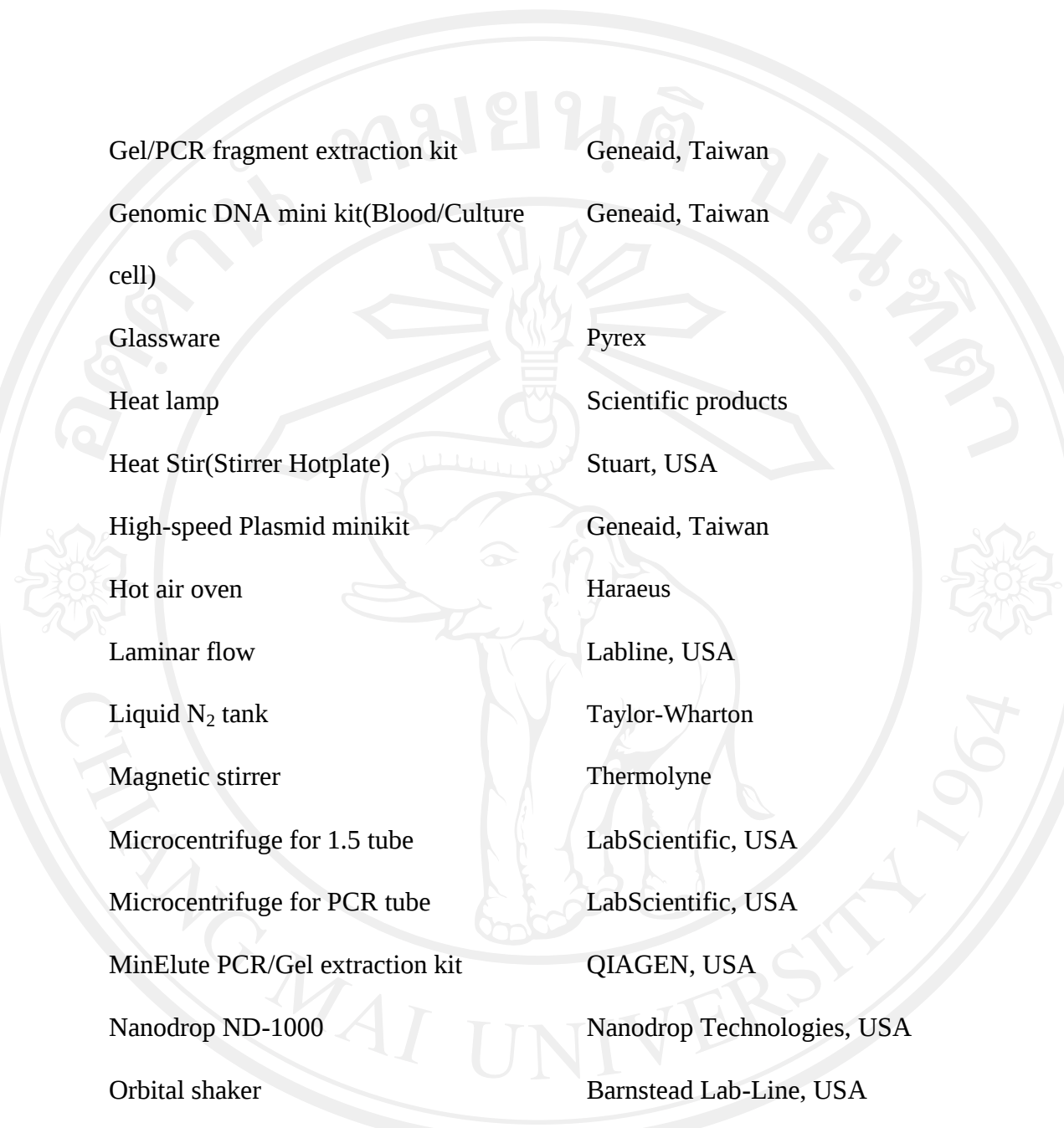


Paraformaldehyde	Merck, Germany
Pfu Polymerase	Promega, USA
Phenol	Pierce, USA
Potassium acetate(CH_3COOK)	Merck, Germany
Potassium chloride(KCL)	Carlo Erba, USA
Potassium dihydrogen phosphate(KH_2PO_4)	Carlo Erba, USA
Potassium hydrogen carbonate(KHCO_3)	Carlo Erba, USA
Potassium hydroxide(KOH)	Merck, Germany
Proline	Sigma-Aldrich, USA
Restriction enzyme	NEB, USA
RPMI-1640	Gibco, USA
Sodium chloride(NaCl)	Sigma-Aldrich, USA
Streptomycin	M&H manufacturing
Sodium dodecyl sulfate(SDS)	USB, USA
T4 DNA ligase	Promega, USA
Taq DNA polymerase	Promega, USA
Tris	Pacific Science, USA
Trypsin	Promega, USA
Tryptophan	Sigma-Aldrich, USA
Xylene cyanol	USB, USA
Yeast extract	Criterion, USA

APPENDIX B

List of instrument used in this study

Instruments and equipments	Company
1.5 ml microcentrifuge tube	Sorensen, USA
1 and 10 ml sterile syringe	Scientific Industries, USA
175 cm ² cell culture flask	Corning, USA
37°C incubator	Heraeus Holding GmbH
50 ml centrifuge tube	Corning, USA
Automatic pipette	Gilson, USA
Block Heater SBH130D	Stuart, USA
Centrifuge	Hettich Holding GmbH, USA
Centrifuge 5417R	Eppendorf, USA
Centrifuge 5810R	Eppendorf, USA
Class II Biological Safety Cabinets	NUAIRE, USA
CO ₂ tank	TSG Gas Services
Cover glasses	Menzel-Glazer, USA
Cryotube	Thermo Fisher, USA
Deionized water machine, NanoPure	Thermo Fisher, USA
Distilled water machine, NanoPure	Hamilton Laboratory
Duran sterile bottle	Duran Group GmbH
Gel electrophoresis machine	Cosmo Bio Co., Ltd.



Gel/PCR fragment extraction kit	Geneaid, Taiwan
Genomic DNA mini kit(Blood/Culture cell)	Geneaid, Taiwan
Glassware	Pyrex
Heat lamp	Scientific products
Heat Stir(Stirrer Hotplate)	Stuart, USA
High-speed Plasmid minikit	Geneaid, Taiwan
Hot air oven	Haraeus
Laminar flow	Labline, USA
Liquid N ₂ tank	Taylor-Wharton
Magnetic stirrer	Thermolyne
Microcentrifuge for 1.5 tube	LabScientific, USA
Microcentrifuge for PCR tube	LabScientific, USA
MinElute PCR/Gel extraction kit	QIAGEN, USA
Nanodrop ND-1000	Nanodrop Technologies, USA
Orbital shaker	Barnstead Lab-Line, USA
Parafilm	Pechiney Plastic Package
Pasteur pipette	Pyrex
Petri-dish, plastic 90 mm	Sterilin, USA
pH meter B200	Eutech Cybermetics
Pipette Boy	Stripettor Costar
Pipette tips	Sorensen, Bioscience



QIAprep Spin miniprep kit	QIAGEN, USA
QIAquick gel extraction kit	QIAGEN, USA
QIAquick PCR purification kit	QIAGEN, USA
Refrigerator	Sharp
Refrigerator-20°C	Brandt
Refrigerator-80°C	Forma Scientific
Refrigerator-20°C	REVCO
Speed Vac system	Thermo Savant
Vortex mixture	Scientific Industries
Water bath	Labline, USA
Water bath	Memmert
Water bath shaker G76D	Brunswick Scientific

APPENDIX C

Preparation of some reagents and buffers

Incomplete RPMI 1640 medium

RPMI 1640	package (13.5 g)
HEPES	2.60 g
NaHCO ₃	3.7 g
Penicillin G	0.0625 g
Streptomycin	100

Add distilled water to 1 liter and adjusted pH to 7.4 and sterile by Millipore filtermembrane (0.2 μ M)

Complete RPMI 1640 medium

RPMI 1640	90	ml
Fetal bovine serum	10	ml

Freezing solution

Fetal calf serum	9.2	ml
DMSO	0.8	ml

Stored at 4°C

10X Phosphate buffer saline (PBS pH 7.4)

NaCl	80	g
KCl	2	g
Na ₂ HPO ₄	14.4	g
KH ₂ PO ₄	2.4	g
DW	800	ml

Adjust pH 7.4 using 1 N NaCl then adjust volume to 1 liter

1X Phosphate buffer saline (PBS pH 7.4)

Phosphate buffer saline 10X	100	ml
DW	900	ml

SDS-PAGE

30% T, 2.7% C Bis-Acrylamide

Acrylamide	29.2	g
Bisacrylamide	0.8	g
DW to	100	ml

Filtrate through 0.45 μ M filter and keep at 4 °C (within 1 month)

0.5 M Tris-HCl pH 6.8

Tris	6.055	g
DW	80	ml

Adjust pH to 6.8 with HCl then adjust volume to 100 ml and kept at 4 °C

1.5 M Tris-HCl pH 8.8

Tris	18.165	g
DW	80	ml
Adjust pH to 8.8 with HCl then adjust volume to 100 ml and kept at 4 °C		

10% Sodium dodecyl sulfate (SDS)

SDS	10	g
DW	100	ml
Keep at room temperature		

10% ammonium persulfate (APS) (preparation before use)

APS	0.1	g
DW	1	ml

Separating gel preparation (12% gel)

DW	3.30	ml
1.5 M Tris-HCl pH 8.8	2.5	ml
10% SDS	100	μl
Acrylamide/Bis (30% T, 2.7% C)	4	ml
10% APS	100	μl
TEMED	5	μl
Total	10.005	ml

Add APS and TEMED into the solution after completely set the gel apparatus

Stacking gel preparation (4% gel)

DW	6.10	ml
0.5 M Tris-HCl pH 8.8	2.50	ml
10% SDS	100.00	μ l
Acrylamide/Bis (30% T, 2.7% C)	1.30	ml
10% APS	100.00	μ l
TEMED	10.00	μ l
Total	10.11	ml

Add APS and TEMED into the solution after completely set the gel apparatus

0.5% Bromophenol blue

BPB	0.05	g
DW	10	ml

SDS reducing buffer

Stock sample buffer	0.95	ml
2-mercaptoethanol	0.05	ml

(add immediately before use)

Total 1 ml

Electrode buffer (1X Running buffer) (0.025 M Tris, 0.192 M glycine, 0.1% w/v SDS, pH 8.3)

Tris-base	3	g
Glycine	14	g
10% SDS	10	ml
DW to	1000	ml
Keep at 4 °C		

Tris-buffered saline (TBS) pH 7.5 (100 mM Tris-HCl, 0.9% w/v NaCl)

Tris	12.1	g
NaCl	9	g
Distilled water	800	ml

Adjust pH to 7.5 then adjust volume to 1000 ml keep at 4 °C

LB(Luria Bertani) culture medium

Tryptone	10	g
Yeast extract	5	g
NaCl	10	g
Distilled water	1000	ml

Autoclaved at 120°C for 15 min, stored at room temperature

LB(Luria Bertani) culture agar

Tryptone	10	g
Yeast extract	5	g
NaCl	10	g
Agar	15	g
Distilled water	1000	ml

Autoclaved at 120°C for 15 min, stored at room temperature

Ampicillin(100 mg/ml)

Ampicillin sodium salt	5	g
Distilled water	50	ml

Filter sterilized through a 0.22 μ m filter and aliquoted into 1.5 ml microcentrifuge tube(1 ml/tube) before stored at -20°C.

Kanamycin(50 mg/ml)

Kanamycin salt	2.5	g
Distilled water	50	ml

Filter sterilized through a 0.22 μ m filter and aliquoted into 1.5 ml microcentrifuge tube(1 ml/tube) before stored at -20°C.

Streptomycin(50 mg/ml)

Streptomycin salt	2.5	g
Distilled water	50	ml

Filter sterilized through a 0.22 μm filter and aliquoted into 1.5 ml microcentrifuge tube(1 ml/tube) before stored at -20°C.

M9 salt

$\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$	64	g
KH_2PO_4	15	g
NaCl	2.5	g
NH_4Cl	5	g
Distilled water	1000	ml

Autoclaved at 120°C for 15 min, stored at room temperature

M9 minimal media

M9 salt	200	ml
Steriled 1M MgSO_4	2	ml
20% glucose	20	ml
Steriled 1M CaCl_2	100	μl
Distilled water	1000	ml

Autoclaved at 120°C for 30 min, stored at room temperature

TAE electrophoresis buffer

Tris	387.6	g
0.5 M EDTA(pH 8.0)	160	ml
100% acetic acid	91.5	ml
Distilled water	8000	ml

Stored at room temperature

Glycerol stock of Bacteria

100% glycerol	0.15	ml
Overnight bacteria culture	0.85	ml

Stored at -80°C

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

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CURRICULUM VITAE

Name

Mr. Kitsanapong Reaksudsan

Date of Birth

June 15, 1988

Education

March, 2000

Certificate of junior high school

Montfort collage, Chiang Mai, Thailand

March, 2006

Certificate of senior high school

High school at Montfort collage, Chiang Mai, Thailand

March, 2010

Bachelor of Science (Medical Technology), Faculty of

Associated Medical Science Chiang Mai University,

Chiang Mai, Thailand

Scholarship

Thailand Graduate Institute of Science and Technology(TGIST) 2010-2012