

CHAPTER II

EQUIPMENTS AND REAGENT

2.1 Instruments

(-20°C) Freezer (Krungthai Ltd., Thailand)

(-80°C) Bio-freezer (Bara Laboratory Co. Ltd., Thailand)

Autoclave:-

- Autoclave HA-30 (Hirayama Manufacturing Co., Japan)
- Autoclave model # LS-2D (Rexall Industries Co. Ltd., Thailand)

Automatic micropipette P2, P10, P20, P100, P200, P1000 (Gilson Medical

Electrical S.A., France)

Balance: Satorius 1702 (Scientific Promotion Co.)

Centrifuge:-

- Centrifuge 5804 R (eppendorf, Germany)
- Centrifuge 5417 C (eppendorf, Germany)
- High speed microcentrifuge MC-15A (Tomy-seiko, Japan)
- High speed microcentrifuge model 1120 (Kubota Corporation, Japan)
- Microcentrifuge model SD220VAC (CE, Taiwan)
- Minicentrifuge (Costar, U.S.A.)

Dry bath incubator (Major Science, Taiwan)

Electronic balance: Alsep EY220A (A&D Co. Ltd., Japan)

Gel documentation system: GeneCam FLEX1 (SYNGENE, EEC) / Digital

graphic printer UP-D895 (Sony Corporation, Japan)

Gene pulser (Bio-Rad Laboratories, U.S.A.)

Hand-held engraving device (Super Tool, Arlec)

Heating block 1761G-26 (Sybron Thermermolyne Co., U.S.A.)

Horizontal gel electrophoresis apparatus:-

- Taitec Pico-2 (Taitec Corporation, Japan)
- MGU-402T (C.B.S. & Scientific Company, U.S.A.)
- GelMate 2000 (TJTTP: Thailand-Japan Technology Transfer Project)

Incubator BM-600 (Memmert GambH, Germany)

Incubator shaker (Innova 4080TM, New Brunwick Scientific, U.S.A.)

Laminar flow: HT123 (ISSCO, U.S.A.)

Light box 2859 SHANDON (Shandon Scientific Co. Ltd., England)

Magnetic stirrer M21/1 (Franz Morat KG GambH, Germany)

Magnetic stirrer and heater (Fisher Scientific, U.S.A.)

MicroPulser (BIO-RAD, U.S.A.)

Microwave oven TRX 1500 (Turbora International Co. Ltd., Korea)

PCR workstation model #P-036 (C.B.S. Scientific Co., U.S.A.)

pH meter model PHM 95 (Radiometer, Denmark)

Phosphor-Imager 400S (Molecular Dynamics, UK)

Power supply:-

- Power supply EC135-90 (E-C Apparatus Corporation, U.S.A.)
- Power Pac 300 (BIO-RAD Laboratories, U.S.A.)
- Power supply EPS 250 (C.B.S. & Scientific Company, U.S.A.)

Real-time detection system:-

- iCycler iQ™ Real-time detection system (Bio-Rad, U.S.A.)
- Rotorgene 3000 Thermal Cycler (CorbettResearch, Sydney, Australia)

Refrigerated microcentrifuge MIKRO 22R (Hettich Zentrifugen, Germany)

Spectrophotometer DU650 (Beckman, U.S.A.)

Stereomicroscope (Olympus, Germany)

Stirrer/Hotplate model PC-320 (Corning, U.S.A.)

Thermal cycler:-

- DNA Engine MJ Research PTC-200 Peltier Thermal Cycler
 (ITOCHU Corporation, Thailand-Japan Technology Transfer Project)
- Mastercycle gradient (eppendorf, Germany)
- GeneAmp PCR System 2400 (Perkin Elmer, U.S.A.)
- iCyclerTM Thermal Cycler/ iCyclerTM Optical Module (BIO-RAD, U.S.A.)

Touch mixer model 232 (Fisher Scientific, U.S.A.)

Ultrasonicator model 28H (Ney Dental Inc., U.S.A.)

Ultrasonic homogenizer (SONOPULS, BANDELIN, Germany)

Ultraviolet Transilluminator:-

- UV Transilluminator model TVC-312R/F (Spectronics Corporation, U.S.A.)
- UV Transilluminator model 2001 microwave (San Gabriel California, U.S.A.)

Vistra FluoroImager (Amersham, UK)

Votex Genie-2 model G560E (Scientific Industries Inc., U.S.A.)

Water bath:-

- Water bath model M20S (Lauda, Germany)
- Water bath model SH28L (Polyscience, U.S.A.)

2.2 Inventory supplies

Disposable pestles (Sigma, U.S.A.)

Hybond N+ nylon membranes (Amersham, UK)

iCycler iQTM Optical Tape (BIO-RAD, U.S.A.)

iCycler iQTM PCR Plates, 96 well (BIO-RAD, U.S.A.)

Microcentrifuge tubes 0.5, 1.5 ml (Axygen Hayward, U.S.A.)

Microcentrifuge tubes 0.5 ml and 1.5 ml (Bio-RAD Laboratories, U.S.A.)

Pipette tips 10, 200, 1000 µl (Axygen Hayward, U.S.A.)

Thin-wall microcentrifuge tubes 0.2 ml (Axygen Hayward, U.S.A.)

2.3 Chemicals

100 base pair DNA Ladder (Promega Co., U.S.A.)

100 mM dNTPs: dATP, dCTP, dGTP and dTTP (Boehringer Mannheim,

Germany)

Absolute ethanol (Merck, Germany)

Acrylamide (Merck, Germany)

Agarose, Seakem LE (FMC Bio products, U.S.A.)

Ammonium persulfate (Merck, Germany)

Beta-mercaptoethanol (Fluka, Switzerland)

Bromphenol blue (Merck, Germany)

Chloroform (Merck, Germany)

Coomassie brilliant blue R-250 (Sigma Chemical Co., U.S.A.)

Diethyl pyrocarbonate sulfoxide (DEPC) (Sigma Chemical Co., U.S.A.)

DyNAzymeTM II DNA Polymerase (Finnzymes, Finland):-

- DNA polymerase
- 10X Mg²⁺ free buffer
- 50 mM MgCl₂ solution

Ethidium bromide (Sigma Chemical Company, U.S.A.)

Ethylene diamine tetraacetic acid, disodium salt dihydrate (Fluka, Switzerland)

GeneAmp PCR core reagent (GibcoBRL, U.S.A.):-

- Taq DNA polymerase
- 10X PCR buffer
- 50 mM MgCl₂ solution

Hydrochloric acid (Merck, Germany)

Isoamyl alcohol (Merck, Germany)

Isopropanol (Merck, Germany)

iQTM SYBR® Green Supermix (BIO-RAD, U.S.A.)

Methanol (Merck, Germany)

N, N'- methylene-bis-acrylamide (Merck, Germany)

Phenol saturated solution (Amresco, U.S.A.)

RNase inhibitor (Bio Basic Inc., Canada)

Sodium acetate (Merck, Germany)

Sodium carbonate (Merck, Germany)

Sodium chloride (Merck, Germany)

Sodium dodecyl sulfate: SDS (Sigma Chemical Company, U.S.A.)

Sodium hydroxide (Merck, Germany)

Tris-(hydroxyl methyl)-aminomethane (Fluka, Switzerland)

TriReagent® (Molecular Research Center, Inc., U.S.A.)

TriZol Reagent (GibcoBRL Life Technologies Inc., U.S.A.)

Xylene cyanol (Sigma, U.S.A.)

2.4 Oligonucleotide primers

- Bio Basic Inc., Canada
- Bio service Unit, Thailand
- Biosynthesis Inc., U.S.A.
- Promega Coperation, U.S.A.

2.5 Enzymes

Ampli Taq DNA polymerase (Perkin Elmer Cetus, U.S.A.)

DyNazymeTM II DNA polymerase (Finnzymes, Finland)

Omniscript Reverse Transcriptase (Qiagen, germany)

Pfu DNA polymerase (Promega Corporation Medison, U.S.A.)

Proteinase K (Life Technologies, Inc., U.S.A.)

RNase Free DNase I (Promega Corporation Medison, U.S.A.)

RNase A (Sigma Chemical Co., U.S.A.)

Taq DNA polymerase (GibcoBRL Life Technologies Inc., U.S.A.)

T4 DNA ligase (Amersham Biosciences, U.S.A.)

2.6 Kits

Dynabeads® Oligo (dt)₂₅ (DYNAL, Norway)

NucleoSpin® Gel Extraction Kit (Macherey-Nagel, Germany)

NucleoSpin® Plasmid DNA Purification Kit (Macherey-Nagel, Germany)

Omniscript RT Kit (Qiagen, Germany)

pGEM®-T Easy Vector Systems (Promega, U.S.A.)

Rediprime TM II random prime labeling system (Amersham Pharmacia Biotech,

UK)

RNeasy Mini Kit (Qaigen, Germany)

SuperScriptTM II Reverse Transcriptase Kit (Invitrogen, U.S.A.)

Total RNA Isolation Mini Kit (Agilent, U.S.A.)

QIAprep Miniprep Kit (Qiagen, Germany)

QIAquick Gel Extraction Kit (Qiagen, Germany)

2.7 Software

BlastX (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi)

Clustal X (Thompson, 1997)

GENETYX (Software Development Inc.)

Genomic Scaffolds (http://www.hgsc.bcm.tmc.edu/projects/honeybee/) at the

Baylor College of Medicine

Interactive Fly (www.sdbonline.org)

Online Mendelian Inheritance in Man (www.ncbi.nlm.nih.gov)