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Appendix

APPENDIX I

CHEMICAL AGENTS AND INSTRUMENTS

A. Chemical substances

Agarose (GIBCO; Grand Island, N.Y. USA)

Bromphenol blue (Sigma, MO, USA)

BsaBI restriction enzyme (New England Biolaboratories Inc., Beverly, MA)

100 bp DNA ladder (Promega, USA)

dNTPs (Promega, USA)

Ethanol (C_2H_5OH) (Sigma. MO, USA)

Ethidium bromide (Sigma. MO, USA)

Filcoll 400 (Sigma, MO, USA)

MMLV-Reverse Transcriptase (Promega, USA)

Msp I restriction enzyme (New England Biolaboratories Inc., Beverly, MA)

Na_2EDTA (Sigma, MO, USA)

PhiX174 DNA/Hae III marker (Promega, USA)

QIAamp Spin Columns (QIAGEN Gmbh, Germany)

QIAquick spin columns (QIAGEN Gmbh, Germany)

Taq DNA polymerase (Promega, USA)

B. Instruments

Agarose submarine gel apparatus

Automatic pipette (Gilson, Lyon, France)

Analytical balance

Collection tubes (2 ml)

Electrophoresis power supply (Biorad, CA, USA)

Glover, sterile

2-ml collection tubes

Incubator (Forma Scientific, Ohio, USA)

Microcentrifuge (Eppendorf, USA)

Mixer-Vertex-Genic (Scientific industries, N.Y., USA)

Pipette tip

PCR machine GeneAmp PCR System 9600 (Perkin elmer)

UV trans-illuminator (ULTRA-LUM, Carson, California)

Water bath

APPENDIX II

REAGENTS AND PREPARATIONS

1). Reagents for genomic DNA extraction

1.1 QIAGEN Protease Stock solution

Add 7 ml of distilled water to the lyophilized QIAGEN protease in the 250-prparation QIAamp kits.

1.2 Buffer AL

Prepare buffer AL by decanting all of reagent AL1 into buffer AL (Reagent AL2). Mix thoroughly by shaking.

1.3 Buffer AW (store at room temperature)

Buffer AW is supplied as a concentrate. Before using for the first time, add the 190-ml of ethanol (96-100%) to buffer AW concentrate as indicated on the bottle. Final buffer volumes are 271 ml.

1.4 Buffer AE (Ready to used)

2. Reagents for PCR product purification

2.1 Buffer PB (Ready to used)

2.2 Buffer PE

Buffer PE is supplied as a concentrate. Before using for the first time, add the 55-ml of ethanol (96-100%) to buffer AW concentrate as indicated on the bottle.

3). Reagents for agarose gel electrophoresis

3.1. 50X Tris-acetate buffer (TAE)

Tris-base 242.0 g

Glacial acetic acid 57.1 mL

0.5 M EDTA pH 8.0 100 mL

Adjust the volume to 1 liter with deionized distill water and sterilize by autoclaving at 121 °C for 15 min

3.2. Working Electrophoresis buffer (1x TAE)

Stock 50XTAE	10	mL
Distill water	490	mL

3.3. 10 mg/ml Ethidium bromide (Et-Br) (Stock)

Ethidium bromide 1 g

DDW 100 mL

Stir on a magnetic stirrer for several hours to ensure that dye has dissolved. Wrap the container in aluminum foil or transfer to a dark bottle and stores at 4 °C

3.4 TAE for Agarose gel preparation

1X TAE	1000	mL
Stock 10 mg/ml Et-Br	5	µL

3.3. 4 % Agarose gel

Agarose ultrapure	4	g
1x TAE with Et-Br	100	mL

3.3. 6x loading buffer

Ficoll 400	20 %
Na2EDTA, pH 8.0	0.1 M
Bromphenol Blue	0.25 %

BIOGRAPHY

Mr. Somboon Nookhai was born on November 9, 1970 in Suphanburi province, Thailand. He graduated with the Bachelor degree of Science in Medical Technology from the Faculty of Medicine, Chulalongkorn University in 1993. Since 1994 to present, he is working at the Thai Red Cross AIDS Research Centre, Bangkok.