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APPENDICES I

Chemical agents and Instruments

A. Chemical agents

Absolute Methanol GR (E. Merck, Darmstadt., Germany)

Agarose (FMC Bioproducts, USA)

Acetone (E. merck., Darmstadt., w. Germany)

Boric acid (Sigma, Mo., USA)

Chloroform (Sigma, MO, U.S.A.)

Diethyl Pyrocarbonate (Sigma, MO, U.S.A.)

dNTP (Promega, WI.,USA)

Ethylene diamine (Sigma, MO, U.S.A.)

Guanidine thiocyanate (Sigma, MO, U.S.A.)

Glacial acetic acid AR (E. merck., Darmstadt., w. Germany)

8-Hydroxyquinoline (Sigma, MO, U.S.A.)

Hydrochlloric Acid (E. merck., Darmstadt., w. Germany)

Isoamyl alcohol (Sigma, Mo., USA)

Isopropanal (Sigma, Mo., USA)

Laury sulfate (Sigma, Mo., USA)

2-Mercaptoethanal (Sigma, Mo., USA)

Mineral oil (Sigma, Mo., USA)

Proteinase-K (Borhring Mannheim, W. germany)

Phenal Equilibrated (Sigma, Mo., USA)

Sodium chloride AR (E.merk. Darmstadt., Germany)

Sodium acetate AR (E.merk, Darmstadt., Germany)

Trisma base (Sigma, Mo., USA)

Yeast tRNA (Gibco, Grand Island.,N.Y.USA)

B. Nucleic acid isolation

Nucleic acid isolation Kit (NucliSens , Organon Teknika, Boxtel, Netherlands)

QIAquick PCR Purification kit (QIAGEN, CA , USA)

C. Enzymes and molecular markers.

Taq DNA Polymerase (Promega, WI.,USA)

Rnasin Ribonuclease Inhibitor (Promega, WI.,USA)

M-MLV Reverse Transcriptase (Promega, WI.,USA)

123 bp molecular marker (Gibco, Grand Island.,N.Y.USA)

100 bp molecular marker (Promega, WI.,USA)

D. Instruments

Automatic pipette, P10/P20/P200/P1000 (Gilson, France)

Microcentrifuge tubes (Eppendorf, USA)

Refrigerated Microcentrifuge (Eppendorf, USA)

Water Bath (Precision, Scientific., USA)

Mixer Vortex-Genic (Scientific., USA)

Larminar Air Flow (Faster, Italy)

PH meter (Orion, USA)

Analytical balance (Satorious, Germany)

Horizontal gel electrophoresis (C.B.S, Scienctific., USA)

Refrigerator (-70^{O} c) (Revco, USA)

Refrigerator (-30^{O} c) (Revco, USA)

Refrigerator (4^{O} c) (Puffer hubbard, USA)

DNA lyophilizer (Savant, USA)

Poraroid camera (FCR-10, Fotodyne, USA)

480 DNA thermocycler (Perkin Elmer, USA)

Test Tube Heater (Stuast Scientific, United Kingdom)

E. Laboratory supplies

Glassware (Pyrex, U.S.A.)

Microcentrifuge tubes 1.5 ml (Costar, England)

Microcentrifuge tubes 0.6 ml (Costar, England))

Filler Pipette tips for P10, P20, P200, P1000 (Treff Lab, Switzerland)

Polaroid films (Polaroid ,USA)

APPENDICES II

REAGENTS

1. 0.01 % DEPC-treated water.

   Added 100 µl of DEPC into 1 litre of distrilled water and left overnight, then autoclaved for 15 minutes at 15 lb/sq inch.

2. Reagent for nucleic acid extraction with Proteinase –K

   -200 mM Tris-HCl PH 7.5

   -25 mM EDTA

   -300 mM NaCl

   -2% w/v SDS

   -200 µg/ml Proteinase-K

   -phenal-chloroform-isoamyl alcohol (25:24:1, v/v/v)

   -Chloroform isoamyl alcohol (24:1,v/v)

   -3 M Na acetate solution

   -Absolute ethanal

   -70 % ethanal

3. Reagent for synthesis cDNA and first round of Multiplex HBV/HCV PCR

   -10x buffer :500 mM KCl, 100 Mm Tris- HCl pH 9.0 at 25 ^{o}c, and 1 % Triton X-100

   -2 mM $MgCl_2$

   -2 mM of each dNTP (dATP, dGTP, dCTP, dTTP)

   -20 pmole/µl Primer TJC1 in DEPC-treated water

   -20 pmole/µl Primer TJC4 in DEPC-treated water

   -25 pmole/µl Primer TJB1 in DEPC-treated water

   -25 pmole/µl Primer TJB2 in DEPC-treated water

   -2.5 units of RNasin

   -2.5 units of M-MLV RT

-2.5 units of *Taq* DNA polymerase

4. Reagent for second round of Multiplex HBV/HCV PCR

-10x buffer :500 mM KCl, 100 Mm Tris- HCl pH 9.0 at 25 ^{o}c, and 1 % Triton X-100

-1.5 mM $MgCl_2$

-2 mM of each dNTP (dATP, dGTP, dCTP, dTTP)

-20 pmole/µl Primer TJC3 in DEPC-treated water

-20 pmole/µl Primer HCV2 in DEPC-treated water

-25 pmole/µl Primer TJB3 in DEPC-treated water

-25 pmole/µl Primer TJB4 in DEPC-treated water

-2.5 units of *Taq* DNA polymerase

4. Reagent for First HBV PCR amplification

-10x buffer :500 mM KCl, 100 Mm Tris- HCl pH 9.0 at 25 ^{o}c, and 1 % Triton X-100

-1.5 mM $MgCl_2$

-0.15 mM of each dNTP (dATP, dGTP, dCTP, dTTP)

-25 pmole/µl Primer TJB1 in water

-25 pmole/µl Primer TJB2 in water

-2.5 units of *Taq* DNA polymerase

5. Reagent for nested HBV PCR amplification

-10x buffer :500 mM KCl, 100 Mm Tris- HCl pH 9.0 at 25 ^{o}c, and 1 % Triton X-100

-1.5 mM $MgCl_2$

-15 mM of each dNTP (dATP, dGTP, dCTP, dTTP)

-25 pmole/µl Primer TJB1 in water

-25 pmole/µl Primer TJB2 in water

-2.5 units of *Taq* DNA polymerase

6. Reagents for agarose gel electrophoresie

-1.5% agarose gel in TBE buffer

-TBE buffer: 0.045 M Tris- borate, 0.001 M EDTA

-6X gel loading solution (0.25 bromophenol blue, 40% (w/v sucrose in water)

-0.2 %(v/v) etheidium bromide solution

-molecular size marker (100 bp. Promega)

BIOGRAPHY

Miss Sineenart Thanomchat was born on 7 September, 1966 in Chaiyapum, Thailand. She graduated with the Bachelor degree of Science in Medical Technology from the Faculty of Associated Medical Science, Khon Kaen University in 1987. Now she works as a medical technologist at the National Blood Center,Thai Red Cross, Bangkok, Thailand.