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สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX I

Chemical agents and Instruments

A. Chemical agents.

- Absolute Ethanol GR (E. merck, Darmstadt., W. Germany)
- Agarose (FMC Bioproducts, USA)
- Boric acid (Sigma, Mo., USA)
- Chloroform (Sigma, Mo., USA)
- Diethyl Pyrocarbonate (Sigma, Mo., USA)
- DNTP (Promega, WI., USA)
- Ethylene diamine tetraacetic acid (Sigma, Mo., USA)
- Ethidium bromide (Sigma, Mo., USA)
- Guanidinium thiocyanate (Sigma, Mo., USA)
- Glacial acetic acid AR (E. merck., Darmstadt., W. Germany)
- 8-Hydroxyquinoline (Sigma, Mo., USA)
- Hydrochloric acid (E.merck, Darmstadt., W.Germany)
- Isoamyl alcohol (Sigma, Mo., USA)
- Isopropanol (Sigma, Mo., USA)
- Lauryl Sulfate (Sigma, Mo., USA)
- 2-Mercaptoethanol (Sigma, Mo., USA)
- Mineral oil (Sigma, Mo., USA)
- Proteinase-K (Boehringer Mannheim, W. Germany)
- Phenol Equilibrated (Sigma, Mo., USA)
- Sodium chloride AR (E.merck, Darmstadt., W. Germany)
- Sodium acetate AR (E. merck, Darmstadt., W. Germany)
- Sodium base (Sigma, Mo., USA)
- Stratagene RNA (Gibco, Grand Island., N.Y.USA)

B. 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)

C. Instruments.

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

Microcentrifuge (Eppendorf, USA)

Refrigerated Microcentrifuge (Eppendorf, USA)

Water Bath (Precision, Scientific., USA)

Mixer Vortex-Genic (Scientific Industries., USA)

Laminar Air Flow (Faster, Italy)

PH meter (Orion, USA)

Analytical balance (Sartorius, Germany)

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

Refrigerator (-70^oc) (Revco, USA)

Refrigerator (-30^oc) (Revco, USA)

Refrigerator (4^oc) (Puffer hubbard, USA)

DNA Lyophilizer (Savant, USA)

Polaroid camera (FCR-10, Fotodyne, USA)

480 DNA thermocycle (Perkin Elmer, USA)

D. Laboratory supplies.

Glassware (Pyrex, USA)

Microcentrifuge tubes 1.5 ml (Costar, England)

Microcentrifuge tubes 0.6 ml (Costar, England)

Filter pipette tips for P10, P20, P200, P1000 (Treff Lab, Switzerland)

Polaroid films (Polaroid, USA)

APPENDIX II

REAGENTS

1. 0.01% DEPC-treated water.

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

2. Reagents for RNA extraction with Proteinase-K

-200 mM Tris-HCl pH 7.5

-25 mM EDTA

-300 mM NaCl

-2% w/v SDS

-200 ug/ml Proteinase-K

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

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

-3 M Na acetate solution

-Absolute ethanol

-70% ethanol

3. Reagents for RNA extraction with Guanidinium isothiocyanate.

-5.75 M GuSCN

-50 mM Tris-HCl PH 7.5

-100 mM 2-mercaptoethanol

-1 ug/ml of yeast tRNA

-isopropanol

70% ethanol

4. Reagents for Reverse Transcriptase.

- 5X RT buffer: 250 mM Tris-HCl pH 8.3, 375 mM KCl, 150 mM MgCl₂, 150 mM DTT.
- 2 mM MgCl₂
- 0.3 mM of each dNTP (dATP, dGTP, dCTP, dTTP)
- 50 pmol/ul Primer 2 in DEPC-treated water
- 20 units of RNasin
- 100 units of M-MLV RT

5. Reagents for First PCR amplification

- 10X buffer: 500 mM KCl, 100mM Tris-HCl PH 9.0 at 25⁰c, and 1% Triton X-100.
- 50 pmol/ul Primer 1 in DEPC-treated water
- 2.5 units of Taq DNA polymerase

6. Reagents for nested PCR

- 10X buffer: 500 mM KCl, 100 mM Tris-HCl PH 9.0 at 25⁰c, 1% Triton X-100
- 2 mM MgCl₂
- 0.2 mM of each dNTP (dATP, dGTP, dCTP, dTTP)
- 50 pmol/ul Primer 3 in DEPC-treated water
- 50 pmol/ul Primer 4 in DEPC-treated water
- 2.5 units of Taq DNA polymerase

7. Reagents for agarose gel electrophoresis

- 1.5% agarose gel in 1X TBE buffer
- TBE buffer: 0.045 M Tris-borate, 0.001 M EDTA
- 6X gel loading dye solution (0.25% bromophenol blue, 40% (w/v) sucrose in water.
- 0.2% (v/v) ethidium bromide solution

-molecular size marker (100 bp, Promega)

-molecular size marker (123 bp, Gibco)



สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

BIOGRAPHY

Miss Tasanee Sakuldamrongpanich was born on March, 1955 in Bangkok, Thailand. She graduated with the Bachelor degree of Science in Medical Technology from the Faculty of Medicine, Chulalongkorn University in 1976. Now she works as a scientist at the National Blood Center, Thai Red Cross Society.



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