

APPENDIX

APPENDIX A

Preparation of reagents

1. Dissolving and neutralization of dNTPs

From dATP, dCTP, dGTP and dTTP powder, each of the dNTPs was dissolved in water and neutralized with NaOH, Tris-HCl (pH 7.2), final concentrations 6 mM and 10 mM, respectively. The solution of 4 mM dATP, 4 mM dCTP, 4 mM dGTP and 4 mM dTTP were obtained. (Steger, unpublished)

The 1mM dNTPs was prepared and stored at -20°C .

2. Ethidium bromide solution

containing :

0.5 $\mu\text{g/ml}$ Ethidium bromide

0.5 x Tris-Boric acid-Ethylenediamine tetraacetic acid (TBE)

The solution was mixed and then stored in a glass tray which was covered with aluminium foil for protection from light and stored at room temperature. This solution was made freshly every two weeks.

3. Loading buffer

containing :

15 % Ficoll (type 400)

1 % Sodium dodecyl sulfate (SDS)

0.3 % Bromophenol blue

0.2 % Xylene cyanol

10 mM Ethylenediamine tetraacetic acid (EDTA)

The solution was stored at room temperature.

4. 10 x PCR buffer

containing :

1 mg/ml Bovine serum albumin (BSA)

15 mM Magnesium chloride (MgCl_2)

500 mM Potassium chloride (KCl)

200 mM Tris-HCl, pH 8.4 (adjusted at room temperature)

0.5 % Tween 20

The solution was stored at -20°C .

5. 0.25 U/ μl *Taq* DNA polymerase

containing :

0.25 U/ μl *Taq* DNA polymerase

50% Glycerol

1x PCR buffer

The solution was mixed and stored at -20°C .

6. Tris-boric acid-ethylenediamine tetraacetic acid (TBE) solution

containing :

89 mM Boric acid (H_3BO_3)

2 mM Ethylenediamine tetraacetic acid (EDTA)

89 mM Tris

The solution was stored at 4°C .

APENDIX B

Equipment and reagents in this study

1. Equipment

| Item | Product Information |
|--|--------------------------------|
| Analytical balance | Shimadzu Corporation |
| Automated DNA thermal cycler | Perkin-Elmer Cetus system 2400 |
| Eppendorf tubes (1.5 ml) | Brand, Germany |
| Freezer to -20°C | Sharp, Japan |
| Gloves, non-sterile | Mala Intertrade Co. |
| Horizontal electrophoresis apparatus | C. B. S. Scientific Co. Ltd. |
| Inverted microscope | Olympus |
| Magnetic stirrer | Scienceware, USA |
| Microcentrifuge | Italy |
| Pipettors, adjustable to deliver 1-1000 μl (P10, P20, P100, P200, P1000) | Gilson, France |
| Pipette tips | |
| Yellow tips for P20-P200 | Brand, Germany |
| white tips for P10 | Treff, Switzerland |
| Thermomixer 5436 | Eppendorf, West Germany |
| Thermostat 5320 | Eppendorf, West Germany |
| Thin-wall PCR tubes (0.2 ml) | Perkin-Elmer Cetus |
| UV-transilluminator | Vilber Lourmat, France |
| Vortex | Scientific Industries, USA |

2. Reagents

| Item | Product Information |
|--|--|
| Agarose | Sigma Chemical Co. Ltd., USA |
| 100 bp ladder | Pharmacia Biotech, USA |
| Boric acid (H_3BO_3) | Sigma Chemical Co. Ltd., USA |
| Bovine serum albumin (BSA) | Sigma Chemical Co. Ltd., USA |
| Chelex-100 chelating resin (sodium form) | Sigma Chemical Co. Ltd., USA |
| dNTPs powder (dATP, dCTP, dGTP, dTTP) | Sigma Chemical Co. Ltd., USA |
| Distilled water (sterile) | Maharaj Nakorn Chiang Mai Hospital, Chiang Mai, Thailand |
| Glycerol | Merck, Germany |
| Ficoll (type 400) | Pharmacia, Sweden |
| Ethylenediamine tetraacetic acid | Merck, Germany |
| Magnesium chloride solution ($MgCl_2$, 25 mM) | Promega, USA |
| Magnesium chloride ($MgCl_2$) | Sigma Chemical Co. Ltd., USA |
| Potassium chloride (KCl) | Merck, Germany |
| Primer A | Bio Service Unit, Department |
| “ B | of Biochemistry, |
| “ C | Mahidol University, |
| and random primers | Bangkok, Thailand |
| Proteinase K | Amresco, OHIO |

2. Reagents (to be continued)

| Item | Product Information |
|-----------------------------------|--|
| Sodium hydroxide (NaOH) | Merck, Germany |
| <i>Taq</i> DNA polymerase | Faculty of Associated Medical Science, Chiang Mai University, Chiang Mai, Thailand |
| Tris (hydroxymethyl) aminomethane | Sigma Chemical Co. Ltd., USA |
| Triton X-100 | Sigma Chemical Co. Ltd., USA |

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