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APPENDICES

APPENDIX I

Reagent for DNA extraction

1. 1 M Tris-HCl (pH 8.0)

Dissolve 121.1 g Tris base in 800 ml of DDW. Adjust the pH to 8.0 by adding 42 ml concentrated HCl. Allow the solution to cool at room temperature before making the final adjustment to the pH with concentrated HCl. Make up the volume of the solution to 1 litter. Dispense into aliquots and sterilize by autoclaving.

2. 0.5 M EDTA

Add 186.1 g of disodium ethylene diamine tetraacetate.$2H_2O$ to 800 ml of DDW. Stir vigorously on a magnetic stirrer. Adjust the pH to 8.0 with NaOH (20 g of NaOH pellets). Dispense into aliquots and sterilize by autoclaving. The disodium salt of EDTA will not go into solution until the pH of the solution is adjusted to approximately 8.0 by the addition of NaOH.

3. 1 M KCl

Dissolve 74.55 g of KCl in 800 ml of DDW. Adjust volume to 1 liter. dispense into aliquots and sterilize by autoclaving.

4. 1 M Mg Cl_2

Dissolve 95.3 g of Mg Cl_2 in 800 ml of DDW. Adjust volume to 1 liter. Dispense into aliquots and sterilize by autoclaving.

5. Lysis buffer

200 μg/ml Proteinase K

0.5% Nonidet P-40

0.5% Tween 20

50 mM KCl

10 mM Tris-HCl (pH 8.0)

3 mM $MgCl_2$

Preparation (10 ml)

Proteinase K	0.2	mg
Nonidet P-40	0.05	ml
Tween 20	0.05	ml
1 M KCl	0.5	ml
1 M Tris-HCl, pH 8.0	0.1	ml
1 M $MgCl_2$	0.03	ml
DDW	9.27	ml

APPENDIX II

Reagents for agarose gel electrophoresis

1. 50x Tris-acetate buffer (TAE)

Tris base	424.0	g
Glacial acetic acid	57.1	g
0.5 M EDTA pH 8.0	100	ml

adjust the volume to 1 liter with DDW and sterilize by autoclaving at 121 °C for 15 min.

2. 10 mg/ml Ethidium bromide

ethidium bromide	1	g
DDW	100	ml

Store in the dark at 4 °C

3. 1.5 % Agarose gel

agarose	0.3	g
1x TAE	20.0	ml
10 mg/ml ethidium bromide	1.0	ml

APPENDIX III

Reagents for dot blot hybridization

1. 20x SSC

NaCl	175.3	g
Sodium citrate	88.2	g
DDW	800.0	ml

Dissolved these components and adjust pH to 7.0 with NaOH (6.5 ml of a 10 N solution). Adjust volume to 1 liter. Dispense into a aliquots. Sterilize by autoclaving.

2. Hybridization buffer (supplied with the kit)

Hybridization buffer	30.0	ml
NaCl	0.88	g
Blocking agent	1.5	g

Dissolved these component into a fine suspension by continue mixing at room temperature for 1 h on magnetic stirrer, then heat to 42°C for 0.5 – 1 h with occasional mixing.

3. Primary wash buffer

SDS	4	g
20x SSC	25	ml

Make up these component to 1 liter. This can be kept for up to 3 month in a refrigerator at 2 - 8°C. Stringency may be increased by using a lower final SSC concentration, for example 0.1x SSC instead of 0.5x SSC.

4. Secondary wash buffer

20x SSC	100	ml

Make up to 1 liter. This can be kept for up to 3 months at 2 - 8°C.

BIOGRAPHY

Miss Khuanjai Ketwong was born on March 25, 1963 in Nakornsawan, Thailand. She graduated with the Bachelor degree of Science in Medical Technology from Chieng Mai University in 1984. Now she works as a Medical technologist at Department of Clinical Pathology, Sawanpracharak Hospital, Nakornsawan.