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APPENDIX I

MEDIA AND REAGENT FOR DNA ISOLATION

1. Modified Hayflick medium

Difco PPLO Broth	750 ml
Horse serum	150 ml
25% (w/v) Difco yeast extract autoclaved 121° C 15 min	80 ml
0.2% Calf DNA	10 ml
Penicillin G 200,000 U	3 ml
10% Thallium acetate	5 ml
0.2% Phenal Red	25 ml
33% Glucose	30 ml

Adjust pH to be 7.8 + 0.2

Store at 4° C

The broth is autoclaved at 121° C 15 minute. The remaining componemts are mixed at room temperature and added to the broth.

2. 10% Sodium dodecyl sulphate (SDS)

Dissolve 10 g of SDS in 90 ml DDW. Heat to assist dissolution. Adjust the volume to 100 ml autoclave 121° C 15 min. Store at room temperature.

3. 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 of concentrated HCl. Allow the solution to cool at room temperature before making the final adjustments to the pH with concentrated HCl. Make up the volume of the solution to 1 liter. Dispense into aliquots and sterilize by autoclaving. If the 1 M solution has a yellow color, discard it and obtain better-quality Tris.

4. 5 M NaCl

Dissolve 292.2 g of NaCl in 800 ml of DDW. Adjust volume to 1 liter. Dispense into aliquots and sterilize by autoclaving.

5. 0.5 mM EDTA (pH8.0)

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 sterilze 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.

6. STE buffer (pH 8.0)

20 mM Tris-HCl (pH 8.0)

10 mM NaCl

10 mM EDTA (pH 8.0)

Preparation (100 ml)

1 M Tris-HCl, pH 8.0	2 ml
5 M NaCl	0.2 ml
0.5 M EDTA, pH 8.0	2 ml
DDW	95.8 ml

7. TE buffer (pH 8.0)

50 mM Tris-HCl (pH 8.0)

10 mM EDTA, pH 8.0

Preparation (10 ml)

1 M Tris-HCl, pH 8.0	0.5 ml
0.5 M EDTA, pH 8.0	0.2 ml
DDW	9.3 ml

APPENDIX II

REAGENTS FOR AGAROSE GEL ELECTROPHORESIS

1. 50XTris-acetate buffer (TAE)

Tris base	424.0 g
Glacial acetic acid	57.1 ml
0.5 M EDTA pH 8.0	100.0 ml

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

2. 10 mg/ml Ethedium bromide

Ethidium bromide	1 g
DDW	100 ml

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

3. 1.5% Agarose gel

Agarose ultrapure (Amresco, U.S.A.)	0.3	g
1X TAE	20.0	ml
10 mg/ml Ethidium bromide	1.0	ul

APPENDIX III

REAGENTS FOR DOT BLOT HYBRIDIZATION

1. 20XSSC

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 the volume to 1 liter. Dispense into a aliquots. Sterilize by autoclaving.

2. Hybridization buffer

5XSSC
0.5% (w/v) Blocking agent (Amersham)
0.1% (w/v) Sodium dodecyl sulphate (SDS)
5% (w/v) Dextran sulphate
100 ug/ml Denatured sheared heterologous DNA

To a half final volume of water add the SSC, SDS and blocking agent, and heat with gentle stirring to 55-60° C. The blocking agent should take 15-30 minutes to dissolve leaving a cloudy solution but with no large particulate matter present. Remove from

the heat, add the dextran sulphate and stir until dissolved which should take a further 15-30 minutes. Make up to a final volume, subaliquot and freeze. Heat denatured (95-100° C for 5 minutes), sheared heterologous DNA can be added before hybridization.

3. Washing buffer

1XSSC, 0.1%(w/v) SDS (Preparation 100 ml)	
20XSSC	5.0 ml
10% SDS	1.0 ml
DDW	94.0 ml
0.5XSSC, 0.1%(w/v) SDS (Preparation 100 ml)	
20XSSC	2.5 ml
10% SDS	1.0 ml
DDW	96.5 ml

Sterilize by autoclaving

4. Antibody Wash buffer

100 mM Tris-HCl (pH 8.0)

150 mM EDTA (pH 8.0)

Preparation (100 ml)

1 M Tris-HCl, pH 8.0	10.0 ml
0.5 M EDTA, pH 8.0	30.0 ml
DDW	60.0 ml

Sterilize by autoclaving

5. Blocking solution (Preparation 100 ml)

Antibody Wash buffer	100 ml
Blocking agent (Amersham)	0.5 g

Dissolve the blocking agent by heating to 55-60° C for approximately 30 minutes. This can be stored at -20° C for several weeks.

6. 0.1%(v/v) Tween20 in Antibody Wash buffer (preparation 100 ml)

Antibody Wash buffer	100 ml
Tween20	0.1 ml

7. 10XSSPE (Preparation 1000 ml)

NaCl	87.0 g
$NaH_2PO_4.H_2O$	13.8 g
EDTA	3.7 g

Dissolved these components and adjust pH to 7.4 with NaOH (6.5 ml of a 10 N solution). Adjust the volume to 1000 ml. Sterilize by autoclaving.

BIOGRAPHY

Miss Sumanee Sirilertpanrana was born on August 13, 1963 in Bangkok, Thailand. She graduated with the Bachelor degree of Science (Medical Technology) from Faculty of Medicine, Chulalongkorn University in 1986. Now she works as a scientist at Department of Microbiology, Chulalongkorn Hospital, Thai red cross society.