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APPENDIX I

MEDIA AND REAGENT FOR DNA EXTRACTION

1. Modified Hayflick medium (18)

Liquid Medium

Difco PPLO Broth	750	ml
Horse serum	220	ml
25% (w/v) Difco Yeast extract autoclaved 121°C 15 min	110	ml
0.2% Calf DNA	10	ml
Penicillin G 200,000 U	3	ml
10% Thallium acetate	5	ml
0.2% Phenol 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 components are mixed at room temperature and added tothe broth.

Solid Medium

This is prepared by adding 1.4 g Purified agar (Code L28, Oxoid Ltd., London) or 0.6-0.8% Noble agar (Difco) to the broth base component before autoclaving.

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 litter. 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 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 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. 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.

7. 1 M $MgCl_2$

Dissolve 95.3 g of $MgCl_2$ in 800 ml of DDW. Adjust volume to 1 liter. Dispense into aliquots and sterilize by autoclaving.

8. 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

9. 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

10. Lysis buffer

500 µg/ml Proteinase K

0.45% Nonidet P-40

0.45% Tween 20

100 mM KCl

20 mM Tris-HCl (pH 8.0)

3 mM $MgCl_2$

Preparation (10 ml)

Proteinase K	0.5	mg
Nonidet P-40	0.045	ml
Tween 20	0.045	ml
1 M KCl	1	ml
1 M Tris-HCl , pH 8.0	0.2	ml
1 M MgCl	0.03	ml
DDW	8.18	ml

APPENDIX II

REAGENTS FOR AGAROSE GEL ELECTROPHORESIS

1. 50Xtris-acetate buffer (TAE)

Tris base	424.0	g
Glacial acetic acid	57.0	ml
0.5 M EDTA pH 8.0	100.0	ml

Agjust the volume to 1 litter with DDW and sterilize by autoclaving at 121 oC 15 min.

2. 10 mg/ml Ethedium bromide

Ethidum 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

BIOGRAPHY

Miss Ajcharaporn Sawatpanich was born on May 24, 1973 in Trad, Thailand. She graduated with the Bachelor degree of Science (Microbiology) from Faculty of Science, Khonkaen University in 1995.