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APPENDICES

APPENDIX I

MEDIAS, REAGENTS, MATERIALS AND INSTRUMENTS

A. MEDIA AND REAGENTS

Absolute ethanol	(Scharlau, Spain)
Agarose (ultrapure)	(GIBCO BRL, USA)
Agarose Low Melting	(USB, UK)
Boric acid	(Bio-Rad, Canada)
Ducitol	(Merck, Germany)
D-Cellobiose	(Sigma, USA)
D-Galactose	(Difco, USA)
D-Xylose	(Merck, Germany)
Ethidium bromide	(USB, UK)
Ethylenediaminetetraacetic acid	(Bio-Rad, Canada)
Glucose	(BDH, UK)
Inositol	(Difco, USA)
λ DNA/*Hind* III Fragments	(GibcoBRL, UK)
Lactose	(Merck, Germany)
Lysing enzyme	(Sigma, USA)
Maleic acid	(Merck, Germany)
Maltose	(Sigma, USA)
McFarland	(bioM´erieux)
Mellibiose	(Sigma, USA)
Noble agar	(Difco, USA)
Phenol, Equilibrated	(USB, UK)
Proteinase K	(USB, UK)
Raffinose	(Difco, USA)
Restriction enzyme *Pst I*	(GibcoBRL, USA)
Restriction enzyme *Sma I*	(Promega, USA)
Rnase solution	(Promega, USA)
RPMI 1640	(Angus, USA)

Sabouraud Dextrose Agar	(Sanofi, France)
Sabouraud Dextrose Broth	(Difco, USA)
Sodium acetate	(USB, UK)
Sodium chloride	(Merck, Germany)
Sodium citrate	(Sigma, USA)
Sodium dodecyl sulphate	(Pharmacia Biotech, Sweden)
Sodium Hydroxide	(Sigma, USA)
Sucrose	(Merck, Germany)
Tris Base	(Promega, USA)
Tween 20	(USB, UK)
2-Mercaptoethanol	(Sigma, USA)
Yeast chromosomal-*S. cerevisiae*	(Bio-Rad, Canada)
Yeast nitrogen base	(Difco, USA)

B. MATERIALS

Eppendrof
Gel block
Micropipett
Plug mold
Test tubge
Tip

C. INSTRUMENTS

Autoclave (model SS-325)	(Tomy seiko, Japan)
Counter-clamped homogenous electric field apparatus	(Bio-Rad, Canada)
Cooling system	(Bio-Rad, Canada)
Electrophoresis chamber	(CBS, USA)
Freezer	(Sunyo, Japan)
Hybridization oven	(Thermo hybraid, USA)
Incubator	(Contherm, New Zealand)
Microcentrifuge	(Hanil, Korea)
Microwave	(Sharp, Japan)

pH meter	(Orion, USA)
Power supply	(CBS, USA)
Pulse-Field Gel Box	(Bio-Rad, Canada)
Pump, Gel molds	(Bio-Rad, Canada)
Refrigerator centrifuge	(Kubota, Japan)
Refrigerator	(Sunyo, Japan)
Rotary shaker	(Bellco Glass, USA)
Vacuum blotter model 780	(Bio-Rad, Canada)
Vortex mixer	(Scientific,USA)
Water bath	(Yamato, Japan)
UV transilluminator	(Bio-Rad, Canada)

APPENDIX II

MEDIA AND REAGENT PREPARATION

A. MEDIA FOR YEAST CULTURE AND IDENTIFICATION

1. Sabouraud Dextrose Agar (Sanofi, France)

Sabouraud dextrose agar powder	65	g
Distilled water	1000	ml

This media was prepared by dissolve the media powder in distill water and mix well. The suspension steriled by autoclave at 121° C , 15 pound/inch^2 pressure, for 15 minutes.

2. Sabouraud Dextrose Broth

Sabouraud Dextrose Broth powder	30	g
Distilled water	1000	ml

This media was prepared by dissolve the powder in distilled water and mix well. The suspension steriled by autoclave at 121° C , 15 pound/inch^2 pressure, for 15 minutes.

3. Glutineous Rice Agar

Glutineous Rice powder	2.5	g
Glucose	10	g
Distilled water	500	ml

The medium was prepared by boil the glutineous rice powder 2.5 g and glucose 10 g in 500 ml distilled water . Use only the supernatant. Add the agar 1.5 g for the solution 100 ml and Tween 80 2-3 drops/100ml. The suspension steriled by autoclave at 121° C , 15 pound/inch^2 pressure, for 15 minutes.

B. REAGENT FOR PLUG PREPARATION

1. 0.5M EDTA (pH 9.0)

Ethylene diaminetetraacetic acid	186.5 g
NaOH	30 g
Deionized water	500 ml

The reagent was made by dissolve 186.5 g of ethylene diaminetetraacetic acid in 900 ml of deionized water, then the pH was adjusted to 9.0 with 1N NaOH. The final volume was bought up to 1000 ml with deionized water. The stock reagent steriled by autoclaving at 121°C, 15 pound/inch^2 pressure, for 15 minutes. The stock reagent was stored at room temperature

2. 1M Tris-HCl (pH 8.3)

Tris base	121.14 g
30% HCl	30-40 ml
Deionized water	1000 ml

This stock reagent was prepared by dissolve 121.14 g of Tris base in 700 ml of deionized water, then the pH was adjusted to 8.3 with conc. HCl. The final volume was bought up to 1000 ml with deionized water. The stock reagent steriled by autocalving at 121°C, 15 pound/inch^2 pressure, for 15 minutes. The stock reagent was stored at room temperature

3. 1M Tris-HCl (pH7.5)

Tris base	121.14 g
30% Hcl	30-40 ml
Deionized water	1000 ml

This stock reagent was prepared by dissolve 121.14 g of Tris base in 700 ml of deionized water, then the pH was adjusted to 7.5 with conc. HCl. The final volume was biught up to 1000 ml with deionized water. The stock reagent steriled by autocalving at 121°C, 15 pound/inch^2 pressure, for 15 minutes. The stock reagent was stored at room temperature

4. 0.5M EDTA (pH7.5)

Na_2EDTA	186.5	g
NaOH	20	g
Distilled water	1000	ml

Dissolve 186.5 g of ethylene diaminetetraacetic acid in 800 ml of deionized water, then the pH was adjusted to 7.5 with 1N NaOH. The final volume was bought up to 1000 ml with deionized water. The stock reagent steriled by autocalving at 121°C, 15 pound/inch^2 pressure, for 15 minutes. The stock reagent was stored at room temperature

5. 0.125M EDTA (pH7 .5)

0.5 M EDTA (pH7.5)	10	ml
Distilled water	30	ml

6. Lysing enzyme solution

a) Lyzing enzyme	2.5	mg
(from Trichoderma harzianum)		
0.05M Tris-HCl (pH7.5)	500	ml
b) 0.05 M EDTA		
0.5M EDTA (pH7.5)	10	ml
Distilled water	90	ml

Suspended 2.5 mg of lyzing enzyme in 0.05M Tris-HCl 500 ml and mix well. The solution is stored at 4° C until use.

7. LMT Agarose 1%

a) LMT agarose	1	g
0.125M EDTA (pH 7.5)	100	ml
b) 0.125M EDTA (pH 7.5)		
0.5 M EDTA (pH 7.5)	10	ml
Distilled water	30	ml

Melting 1 g LMT agarose in 100 ml of 0.125M EDTA by heating. And stored at 4°C until use. Before use this gel is melted by heating. No strerilization.

8. LET buffer (Fresh solution)

0.5M EDTA (pH7.5)	30	ml
1M Tris-HCl (pH7.5)	1.5	ml
2- mercaptoethanol	1.5	ml

9. NDS buffer (Fresh solution)

a) 0.5 M EDTA (pH9.0)	30	ml
1M Tris-HCl (pH8.3)	1.5	ml
SDS	0.018	g
Protease K	5	mg

Mix 0.5M EDTA 30 ml and 1M Tris-HCl (pH8.3) 1.5 ml together. Add 0.018g of SDS and 5 mg of protease K in this solution. Then incubate 60°C in waterbath for 30 minutes before use.

b) 1M Tris HCl (pH8.3)

Tris base	121.14	g
30% HCl	30-40	ml

adjust pH to 8.3 after adding HCl to water

10. 0.05M EDTA (pH9.0)

0.5 M EDTA(pH9.0)	10	ml
Distilled water	90	ml

C. REAGENT AND MEDIA FOR ANTIFUNGAL SUSCEPTIBILITY TEST

1. RPMI1640

RPMI 1640	46.19	g
(Angus, contains 0.165 M MOPS and L-glutamine)		
Glucose	20	g
Agar	15	g
Distilled water	1000	ml

1. Dissolve the RPMI powder in 500 ml deionised water. Adjust the pH to 7.0 with 1 N NaOH.
2. Filter sterilise with a 0.2 μm filter.

3. Dissolve the glucose and agar in 500 ml deionised water. Autoclave for 15 minutes at 15 psi pressure (approx. 121°C) and then cool to approx. 50°C.
4. Gently warm the sterile RPMI + MOPS solution to approximately 45°C and mix it with the cooled glucose-agar solution.
5. Cool the autoclaved agar solution to approx. 45-50°C before pouring.
6. Generally, 60 ml agar solution to is required for a 150 mm petri dish and 25 ml for a 90 mm petridish.
7. Perform quality control for yeast and molds as relevant.

2. 0.85% Normal Saline

NaCl	0.85	g
Distilled water	100	ml

Suspended NaCl 0.85 g in 100 ml distilled water. The stock reagent steriled by autocalving at 121°C, 15 pound/inch^2 pressure, for 15 minutes. This solution was stored at room temperature.

D. REAGENTS AND MEDIAS FOR PLASMID PREPARATION

1. LB (Luria-Bertani) Broth

Bactopeptone	1	g
Yeast Extract	0.5	g
NaCl	0.5	g
Distilled water	100	ml

The medium was prepared by dissolve all ingredients in 100 ml of distilled water. The sterilization of this media was made by autocalving at 121° C, 15 pound/inch^2 pressure, for 15 minutes.

Stock ampicillin (50 mg/ml)

The stock reagent was prepared by add 500 mg of ampicillin sodium in steriled distilled water 10 ml. Afterthat the stock reagent was filtrated by use filter membrane (0.22 micron filter) and stored at –20 °C.

When the LB agar was cool down add the stock of ampicillin (ampicillin : medium = 100 microliters : 100 ml) .

2. LB (Luria-Bertani) Agar

Bactopeptone	1	g
Yeast Extract	0.5	g
NaCl	0.5	g
Agar	1.5	g
Distilled water	100	ml

The medium was prepared by dissolve all ingredients in 100 ml of distilled water. The sterilization of this media was made by autocalving at 121° C, 15 pound/inch^2 pressure, for 15 minutes.

3. 0.7% Agarose

agarose	0.7	g
Distilled water	100	ml

The gel was prepared by dissolve agarose 0.7 g into distilled water 100 ml. The agar was melting by heat. No sterilization.

4. 3M CH_3CooNa

Sodium acetate	49.218	g
Distilled water	200	ml

The solution was prepared by mix 49.218 g of sodium acetae in 200 ml of distilled water and mix well. The solution was stored at room temperature until use.

5. 10% SDS

SDS	10	g
Distilled water	100	ml

This solution was prepared by add SDS 10 g into 100 ml of distilled water and then mixed well. No strilization.

6. Solution I (for 300 ml)

Tris HCL (pH8.0)	7.5	ml
EDTA (pH8)	15	ml
Glucose	2.7	g

Distilled made up to	300	ml

Steriled by autocalving at 121°C, 15 pound/inch^2 pressure, for 15 minutes. This solution was stored at room temperature

7. Solution II (Fresh reagent) (for 100 ml)

10% SDS	10	ml
5N NaOH	4	ml
Distilled water	86	ml

The solution was made by dissolve 10 %SDS 10 ml and NaOH 4 ml in 86 ml of distilled water. This solution was stored at room temperature

8. Solution III

Na.acetate.3H2O	408.1	g
Distilled water	800	ml

Adjust to pH 5.2 with glacial acetic acid and maded up to 1000 ml by distilled water. Sterilized by autocalving at 121°C, 15 pound/inch^2 pressure, for 15 minutes. This solution was stored at room temperature

E. REAGENT FOR PFGE

1. 0.8% Agarose gel

Agarose powder	0.8	g
0.5X TBE	100	ml

Suspended 0.8 g of agarose powder in 0.5X TBE 100 ml. The suspension was melted by heat.

2. 1% Agaose gel

Agarose powder	1	g
0.5X TBE	100	ml

Suspended 1 g of agarose powder in 0.5X TBE 100 ml. The suspension was melted by heat.

3. 10X TBE

Tris base	108	g
Borric acid	55	g
Na_2EDTA	7.44	g
Distilled water	1000	ml

This stock reagent was prepared by dissolve 108 g of Tris base, 55 g of borric acid and 7.44 g of Na2EDTA The final volume was biught up to 1000 ml with deionized water. The stock reagent steriled by autocalving at 121°C, 15 pound/inch^2 pressure, for 15 minutes. The stock reagent was stored at room temperature

4. 0.5X TBE

10X TBE	50	ml
Distilled water	950	ml

The reagent was prepared by mix 50 ml of 10X TBE in distilled water 950 ml.

F. REAGENT FOR SOUTHERN HYBRIDIZATION

1. Denature solution

NaCl	87.8	g
NaOH	20.0	g
Distilled water to	1000	ml

The reagent was prepared by mix all ingredients in distilled water 1000 ml and mix well. Not need to sterile.

2. Neutralizing solution (pH7.0)

Tris base	121.1	g
NaCl	87.0	g
Distilled water to	1000	ml

The reagent was prepared by mix all ingredients in distilled water 1000 ml and mix well. Not need to sterile.

3. 0.25N HCl

HCl concentrated	20.97	ml
Distilled water made up to	1000	ml

The solution was prepared by diluted 20.97 ml of HCl in 1000 ml of distilled water and mix well. Not need to sterile.

4. 10X SSC buffer

NaCl	262.5	g
Trisodium citrate	132.3	g
Distilled water	3000	ml

Add all ingredients in the Distilled water and mix well. The solution was stored in room temperature until use.

5. 2X SSc buffer

10X SSC buffer	100	ml
Distilled water	400	ml

This solution was prepared by diluted 100 ml of 10X SSC buffer in 400 ml of distilled water. The solution was stored in room temperature until use.

6. 2X SSC buffer + 0.1% SDS (1000 ml)

NaCl	17.5	g
Sodium citrate	8.8	g
Distilled water	990	ml

This solution is prepared by mix all ingradient in 990 ml of distilled water. The sterilization was made by autoclave at 121°C, 15 pound/inch^2 pressure, for 15 minutes. After this solution is cool down add 10% SDS 10 ml to this solution.

7. 0.5X SSC buffer + 0.1%SDS

Nacl	4.735	g
Sodium citrate	2.2	g
Distilled water	990	ml

pressure, for 15 minutes. After this solution is cool down add 10% SDS 10 ml to this solution.

8. Washing buffer

Maleic acid	11.067 g
NaCl	8.766 g
Distilled water	1000 ml

Dissolve all ingradent in 800 ml of deionized water, then the pH was adjusted to 7.5 with 1NNaOH. The final volume was bought up to 997 ml with deionized water and add Tween 20 (v/v) 3 ml into this reagent. The stock reagent steriled by autocalving at 121°C, 15 pound/inch^2 pressure, for 15 minutes. The stock reagent was stored at 15-25°C.

9. Maleic acid

Maleic acid	11.607 g
NaCl	8.766 g
Distilled water	900 ml

Dissolve all ingradent in 800 ml of deionized water, then the pH was adjusted to 7.5 with 1NNaOH. The final volume was bought up to 1000 ml with deionized water. The stock reagent steriled by autocalving at 121°C, 15 pound/inch^2 pressure, for 15 minutes. The stock reagent was stored at 15-25°C.

10. Detection buffer

1 M Tris HCl	100 ml
5M NaCl	20 ml
Distilled water made up to	1000 ml

The reagent was prepared by mix all solution together afterthat add the distilled water made up to 1000 ml. The reagent was sterilization by autocalving at 121°C, 15 pound/inch^2 pressure, for 15 minutes. The stock reagent was stored at 15-25°C.

11. TE buffer

Tris	1.2114 g
EDTA	0.372 g
Distilled water	900 ml

Dissolve all ingradent in 900 ml of deionized water, then the pH was adjusted to 8.0 with HCl. The final volume was bought up to 1000 ml with deionized water. The stock reagent steriled by autocalving at 121°C, 15 pound/inch^2 pressure, for 15 minutes. The stock reagent was stored at 15-25°C.

12. 1M Tris HCl (pH 9.5)

Tris base	121.14 g
30% Hcl	30-40 ml
Deionized water	1000 ml

This stock reagent was prepared by dissolve 121.14 g of Tris base in 700 ml of deionized water, then the pH was adjusted to 9.5 .The final volume was biught up to 1000 ml with deionized water. The stock reagent was steriled by autocalving at 121°C, 15 pound/inch^2 pressure, for 15 minutes. The stock reagent was stored at room temperature

13. 5M NaCl

NaCl	58.44 g
Distilled water	200 ml

The solution was prepared by add 58.44 g of NaCl in distilled water 200 ml and mix well. The solution was steriled by autocalving at 121°C, 15 pound/inch^2 pressure, for 15 minutes. The stock reagent was stored at room temperature

14. 0.2M EDTA (pH 8.0)

Ethylene diaminetetraacetic acid	37.224 g
Deionized water	500 ml

The reagent was made by dissolve 37.224 g of ethylaene diaminetetraacetic acid in 400 ml of deionized water, then the pH was adjusted to 8.0. The final volume was bought up to 5000 ml with deionized water. The

stock reagent steriled by autoclaving at 121°C, 15 pound/inch^2 pressure, for 15 minutes. The stock reagent was stored at room temperature.

15. 1X Blocking solution (Fresh solution)

10X blocking solution	10	ml
Maleic acid	90	ml

The reagent was prepared by mix all solution together and mix well. No sterilization.

16. Antibody solution (150 mU/ml) (Freshly prepare)

1X Blocking solution	5	ml
Antibody (750 mU/ml)	0.001	ml

17. Color substrate solution (Freshly prepare)

Detection buffer	10	ml
NBT/BCIP	0.2	ml

BIOGRAPHY

Miss Thida Thaweephon was born in September 25, 1978 in Trat, Thailand. She graduated with the Bachelor degree of Science in Microbiology from Faculty of Science, Burapha University, in 1999.