APPENDIX

1. 0.5X Tris-Acetate-EDTA buffer (TAE)

Tris base	2.42	gm.
Gracial acetic acid	0.571	ml.
0.5 M EDTA (pH 8.0)	1.0	ml.

Dissolve all ingredients in distilled water and fill up to 1,000 ml. Sterilize by autoclaving and keep at room temperature.

2. 1.5% agarose gel

Agarose gel	1.5	gm.
0.5x TAE	100	ml.

Melt in microwave oven for 2 minutes

3. Stock ethidium bromide (10 mg/ml)

Ethedium bromide	1.0	gm

Dissolve in 100 ml distilled water, mix thoroughly and keep in dark bottle at 4 °C.

3.1 Working solution

Dilute stock ethidium bromide to 1:50 in distilled water (final concentration = 0.2% ethidium bromide).

4. 6x gel loading buffer

1 mg/ml (w/v) bromphenol blue

30% (w/v) glycerol

Dissolve all ingredients in distilled water. Mix thoroughly and keep at –20 °C

5. Working 1 kb DNA marker

1 kb Plus DNA Ladder (Gibco BRL, USA)	5	µl.
6x gel loading buffer	10	µl.
Distilled water	45	µl.

Mix thoroughly and keep at –20 °C.

6. 10X Tris/NaCl/EDTA (TNE) buffer stock solution (Chater III, 2.6)

Tris base (MW = 121.14)	12.11	g.
EDTA, disodium salt, dihydrate, (MW = 372.20)	3.72	g.
Sodium chloride, (MW = 58.44)	116.89	g.
Distilled water	to 800	ml.
Concentrated HCl	to pH 7.4	
Distilled water	to 1,000	ml.

Filter before use (0.45 µm). Store at 4 °C for up to 3 months.

7. Assay solution for diluted sample DNA (Chater III, 2.6)

H 33258 stock solution	10	µl.
10X TNE	10	ml.
Distilled filtered water	90	ml.

8. **1M Tris-HCl (pH 7.6)**

Tris-HCl	121.1	gm.
Distilled water	900	ml.
Adjust pH to 7.6 with conc. HCl		
Add distilled water up to	1000	ml.
Sterilize by autoclaving		

9. **0.5 M Ethylenediamine tetraacetic acid (EDTA, pH 8.0)**

EDTA disodium salt	181.1	gm.
Distilled water	900	ml.
Adjust pH to 8.0 with NaOH pellets		
Add distilled water up to	1,000	ml.
Sterilize by autoclaving		

10. **Phosphate buffered saline (PBS) pH 7.0**

10.1 Stock solution (10x PBS)

NaCl	80.0	gm.
KCl	2.0	gm.
Na_2HPO_4	11.5	gm.
KH_2PO_4	2.0	gm.
Add distilled water up to	1,000	ml.

10.2 Working solution

10x PBS	100	ml.
Distilled water	900	ml.

Adjust pH to 7.0, store at room temperature.

11. LB medium/liter (Chapter III)

Tryptone	10	gm.
Yeast extract	5	gm.
NaCl	5	gm.
Distilled water	to 1	liter

Adjust pH to 7.0

For agar plate, add 15 gm. agar /liter

12. Solution I for alkaline lysis method (Chapter III, 2.4)

50 mM glucose

25 mM Tris·Cl (pH 8.0)

10 mM EDTA (pH 8.0)

Sterilize by autoclaving and store at 4 °C

13. Solution II for alkaline lysis method (Chapter III, 2.4)

0.2 N NaOH

1% SDS

Mix thoroughly and keep on ice, prepare fresh.

14. Solution III for alkaline lysis method (Chapter III, 2.4)

5 M potassium acetate	60	ml.
Glacial acetic acid	11.5	ml.
H_2O	28.5	ml.

The resulting solution is 3 M with respect to potassium and 5 M with respect to acetate.

15. SOC medium (100 ml, Chapter III, 2.4)

Tryptone	2.0 g.
Yeast extract	0.5 g.
NaCl	1.0 ml.
1 M KCl	0.25 ml.
2 M Mg^{2+} stock, filter-sterilized	1.0 ml.
2 M glucose, filter-sterilized	1.0 ml.

Add tryptone, yeast extract, NaCl and KCl to 97 ml distilled water. Stir to dissolve. Autoclave and cool to room temperature. Add 2 M Mg^{2+} stock and 2 M glucose. Bring to 100 ml with sterile distilled water. Filter the complete medium through a 0.2 μ filter unit. The final pH should be 7.0.

16. 2 M Mg^{2+} stock (Chapter III, 2.4)

$MgCl_2 \cdot 6H_2O$	20.33 g.
$MgSO_4 \cdot 7H_2O$	24.65 g.

Add distilled water to 100 ml. Filter sterilizes.

17. 1X Washing buffer (Chapter III, 2.7)

0.1 M maleic acid

0.15 M NaCl

Adjust pH to 7.5 with concentrated NaOH, sterilize by autoclaving and store at room temperature.

18. Stock blocking solution (Chapter III, 2.7)

Dissolve blocking reagent in washing buffer to a final concentration of 10% (w/v) with shaking and heating in a microwave oven, sterilize by autoclaving, and store at 4 °C.

18.1 working solution

Dilute stock blocking solution to 1:10 in 1M maleic acid (final concentration = 1% blocking reagent)

19. 20X Sodium chloride sodium citrate buffer (SSC, Chapter III, 2.7)

NaOH	175.3 g.
NaCl	88.2 g.
Sterile water	to 1,000 mL.

Adjust pH to 7.0 with NaOH, sterilize by autoclaving, and store at 4 C.

20. Hybridization solution (Chapter III, 2.7)

1% blocking reagent (w/v)

1% N-lauroylsarcosine (w/v)

0.02% SDS (w/v)

Dissolve all ingredients in distilled water, fill up to 100 ml and keep at 4 °C.

21. Detection buffer (Chapter III, 2.7)

100 mM Tris-HCl

100 mM NaCl

50 mM $MgCl_2$

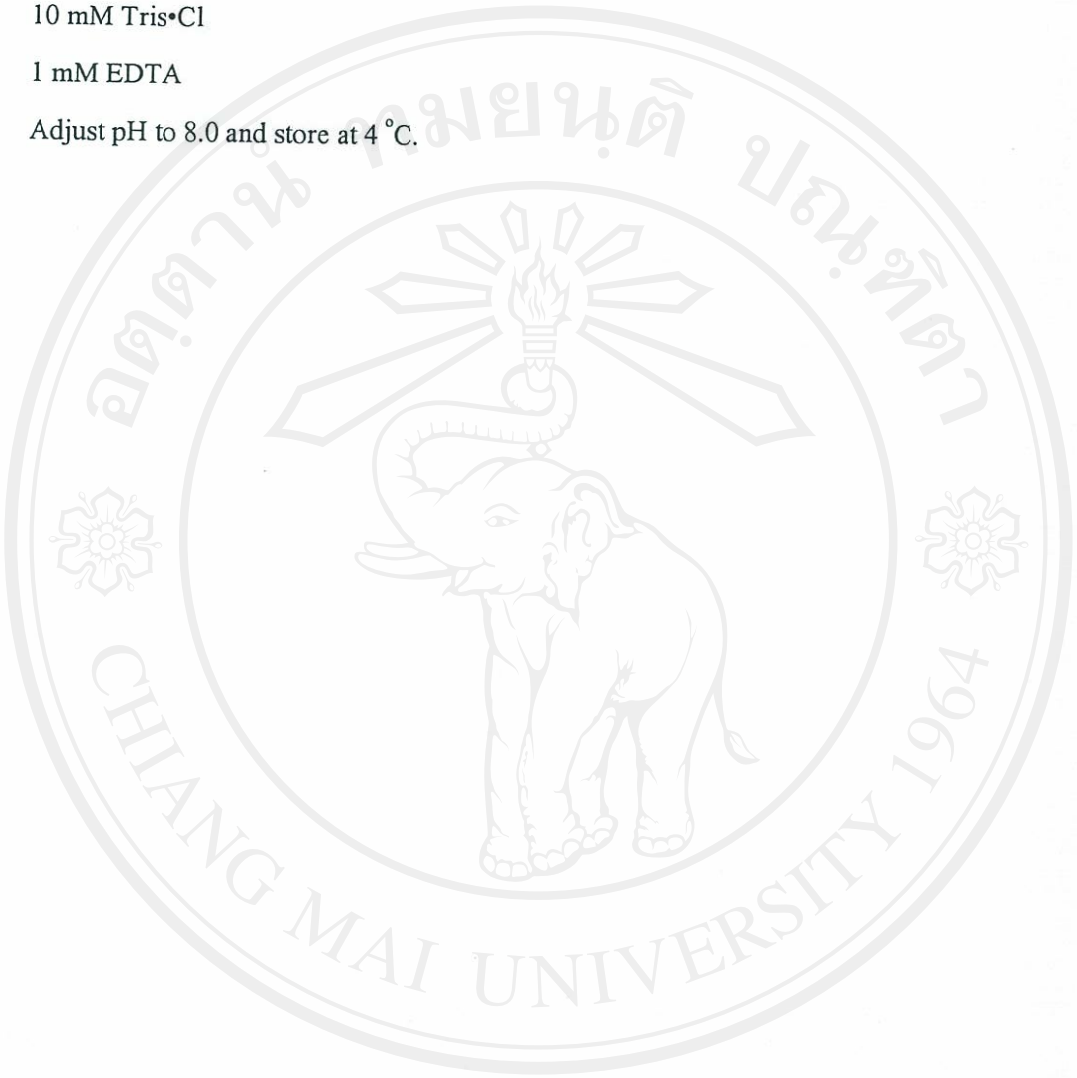
Adjust pH to 9.5 and store at 4 °C.

22. TE buffer (pH 8.0)

10 mM Tris•Cl

1 mM EDTA

Adjust pH to 8.0 and store at 4 °C.



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