APPENDIX

APPENDIX A

Equipments

Equipments	source
1. Balance; Analytical balance	Sartorius, Germany
2. Biological safety cabinet class II	Gelman Science, Australia
3. Centrifuge; Refrigerated centrifuge	Sorvall, Germany
4. Deionization system	ELGASTAT, England
5. DNA thermal cycle	Gene Amp PCR system 2700 Applied Biosystems, Germany
6. Dry block heater	Biosan Laboratory Inc., USA
7. Freezer -20 °C	Sanyo, Thailand
8. Freezer -70 °C	Kelvinator Scientific, USA
9. Horizontal Gel Electrophoresis	Mupid-2, Advance, Japan
10. Hot air oven	Sheldon, USA
11. Microwave	Shap, Japan
12. pH meter	Pierce, USA
13. Photo documentation	Fotodyne, USA
14. Refrigerator 4 °C	Sunyo, Thailand
15. Vortex mixer	Scientific Industries, USA
16. Water bath	Sheldon, USA

APPENDIX B

Chemicals and reagents

Chemicals and reagents	source
1. Absolute ethanol	Merck, Darmstadt, Germany
2. Acetic acid, glacial	Merck, Darmstadt, Germany
3. Agarose	Seakem®, Rockland, ME, USA
4. Ammonium acetate	Fluka BioChemika, Buchs
5. D(+)- Glucose	Merck, Darmstadt, Germany
6. Disodium Ethylenediaminetetra acetate 2 H_2O	Amresco. OH, USA
7. DNA marker: 1 Kb Plus DNA LadderTH	Gibco BRL, NY, USA
8. Ethidium bromide	Sigma, MO, USA
9. Ethyl alcohol	Merck, Darmstadt, Germany
10. Magnesium chloride -6- hydrate	Merck, Darmstadt, Germany
11. Potassium acetate	Merck, Darmstadt, Germany
12. Sodium chloride	Merck, Darmstadt, Germany
13. Sodium hydroxide	Merck, Darmstadt, Germany
14. Tris base	Sigma, MO, USA
15. Tris Hydrochloride	Invitrogen Life technologies, USA
16. Tryptone	Fisher Scientific
17. Tween 20	Organics, USA
18. Yeast extract	GIBCO BRL, UK

APPENDIX C

Reagents and buffer preparation

1. 50X Tris acetate buffer (TAE)

Tris base	242 gm
Glacial acetic acid	57.1 ml
0.5M EDTA (pH 8.0)	100 ml

Dissolve all ingredients in distilled water and fill up to 1,000 ml.

Sterilize by autoclaving and keep at room temperature

2. 0.5X Tris acetate buffer (TAE)

Stock 50X TAE	20 ml
Distilled water	1980 ml

Keep at room temperature

3. 2 % Agarose gel

LE Agarose	2 gm
0.5X TAE	100 ml

Melt in microwave oven for 2 minutes

4. 10 mg/ml Ethidium bromide

Ethidium bromide	1.0 gm

Dissolve in 100 ml distilled water, mix thoroughly and keep in dark bottle at room temperature

5. 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

6. Working 1 kb DNA marker

1 µg/µl 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

7. LB medium/liter

Tryptone	10	gm
Yeast extract	5	gm
NaCl	5	gm

Dissolve all ingredients in distilled water and fill up to 1 liter

Adjust pH to 7.0

8. LB plates with ampicillin

Agar granulated	15	gm
LB medium	1	liter

Sterilize by autoclaving and keep at room temperature until cool down to 50° C, add ampicillin 100 µg/ml for final concentration and keep at 4°C

9. 7.5M Ammonium acetate

Ammonium acetate	77.75	gm
Distilled water	100	ml

Sterilize by filter sterilizing and keep at 4°C

10. Lysis buffer

50 mM KCl
10 mM Tris-HCl
2.5 mM $Mgcl_2$
0.45% NP-40
0.45% Tween-20
Dissolve all ingredients in distilled water, mix thoroughly
Sterilize by autoclaving and keep at 4°C

11. Solution I for alkaline lysis method

50 mM glucose
25 mM Tris-HCl (pH 8)
25 mM EDTA (pH 8)
Sterilize by autoclaving and store at 4°C

12. Solution II for alkaline lysis method

0.2 N NaOH
1% (w/v) SDS
Mix thoroughly and keep on ice, prepare fresh.

13. Solution III for alkaline lysis method

5 M potassium acetate	10	ml
Gracial acetic acid	11.5	ml
Distilled water	28.5	ml

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

CIRRICULUM VITAE

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