

**APPENDICES**

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

Copyright© by Chiang Mai University

All rights reserved

## APPENDIX A

### Equipments

Equipments	source
1. Balance; Analytical balance	Sartorius, Germany
2. Biological safety cabinet class II	Gelman Science, Australia
3. Centrifuge	Denville 260D Scientific Metuchen, USA
4. Centrifuge; Refrigerated centrifuge	Sorvall, Germany
5. Deionization system	ELGASTAT, England
6. DNA thermal cycle	Chromo 4™, BIO-RAD, USA MJ PTC-200, BIO-RAD, USA
7. Dry block heater	Biosan Laboratory Inc., USA
8. Freezer -20 °C	Sanyo, Thailand
9. Freezer -70 °C	Kelvinator Scientific, USA
10. Hot air oven	Sheldon, USA
11. Refrigerator 4 °C	Sunyo, Thailand
12. Vortex mixer	Scientific Industries, USA
13. Water bath	Sheldon, USA

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

Copyright© by Chiang Mai University

All rights reserved

## 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 <sub>2</sub> O	Amresco. OH, USA
7. DNA marker: 1 Kb Plus DNA Ladder <sup>TH</sup>	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

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
Copyright© by Chiang Mai University  
All rights reserved

## APPENDIX C

### Reagents and buffer preparation

#### 1. 2 % Agarose gel

LE Agarose 2 gm

0.5X TAE 100 ml

Melt in microwave oven for 2 minutes

#### 2. 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.

#### 3. 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

#### 4. Luria-Bertai (LB) plates 100 ml

Tryptone 1 gm

Yeast extract 0.5 gm

NaCl 0.5 gm

Agar granulated 1.5 gm

Dissolve all ingredients in distilled water and boil. Sterilize by autoclaving and keep at room temperature until cool down to 54 °C, Pour plate and keep at 4°C.

**5. LB medium 100 ml**

Tryptone	1 gm
Yeast extract	0.5 gm
NaCl	0.5 gm

Dissolve all ingredients in distilled water and boil. Sterilize by autoclaving and aliquot to 20 ml screw cap tube. Store at 4°C.

**6. Lysis buffer for sample preparation 100 ml**

1 M Tris-HCl pH 8.0	5.0 ml
0.5 M EDTA	0.2 ml
Triton X-100	1.0 ml

Dissolve all ingredients in distilled water, mix thoroughly

Sterilize by autoclaving and keep at 4°C

**7. Solution I for alkaline lysis method**

50 mM glucose	4.50 g
25 mM Tris-HCl (pH 8.6)	6.25 ml
10 mM EDTA (pH 8.0)	10.00 ml

Dissolve all ingredients in distilled water then top up to 500 ml. Sterilize by autoclaving and store at 4°C

**8. Solution II for alkaline lysis method**

0.4 N NaOH
2% (w/v) SDS

Mix thoroughly and keep on ice, freshly prepared.

**9. Solution III for alkaline lysis method**

5 M potassium acetate	10 ml
Glacial 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.

**10. 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

**11. 0.5X Tris acetate buffer (TAE)**

Stock 50X TAE	20 ml
Distilled water	1980 ml

Keep at room temperature

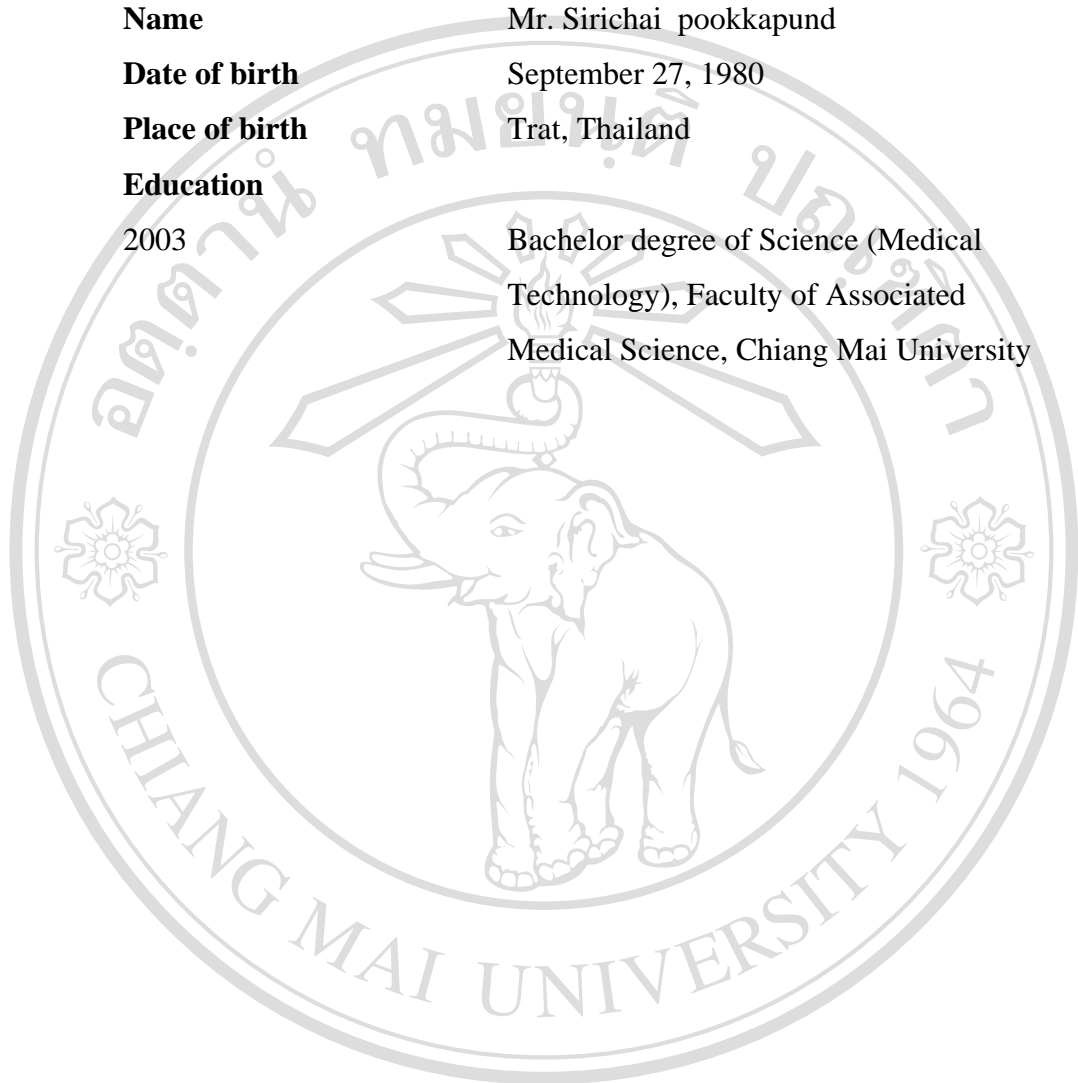
**12. Working 1 kb DNA marker**

1 $\mu\text{g}/\mu\text{l}$ 1 Kb Plus DNA Ladder™ (Gibco BRL, USA)	5 $\mu\text{l}$
6x gel loading buffer	10 $\mu\text{l}$
Distilled water	45 $\mu\text{l}$

Mix thoroughly and keep at  $-20^{\circ}\text{C}$

## CIRRICULUM VITAE

**Name** Mr. Sirichai pookkapund  
**Date of birth** September 27, 1980  
**Place of birth** Trat, Thailand  
**Education**  
2003 Bachelor degree of Science (Medical  
Technology), Faculty of Associated  
Medical Science, Chiang Mai University



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
Copyright© by Chiang Mai University  
All rights reserved