



APPENDIX

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

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APPENDIX A

CHEMICALS AND REAGENTS

Chemicals and Reagents	Source
1 Kb Plus DNA Ladder™	Invitrogen, USA
50 bp DNA Ladder GeneRuler™	Fermentas, USA
Ampicillin	Sigma, MO, USA
Absolute ethyl alcohol	Merck, Darmstadt, Germany
Beta-globin forward and reverse primer	Operon, Cologne, Germany
Boric acid	Merck, Darmstadt, Germany
Bromophenol blue	Merck, Darmstadt, Germany
Disodium ethylenediaminetetra acetate·2H ₂ O	Amresco, OH, USA
Disodium hydrogen phosphate	Merck, Darmstadt, Germany
Ethidium bromide	Sigma, MO, USA
Ethyl alcohol	Merck, Darmstadt, Germany
FastStart Universal Probe Master	Roche Applied Science, Penzberg, Germany
Glycerol	Sigma, MO, USA
High Pure PCR Product Purification Kit	Roche Applied Science, Mannheim, Germany
HPV PGMY11-A forward primer	Operon, Cologne, Germany
HPV PGMY11-B forward primer	Operon, Cologne, Germany
HPV PGMY11-C forward primer	Operon, Cologne, Germany
HPV PGMY11-D forward primer	Operon, Cologne, Germany
HPV PGMY11-E forward primer	Operon, Cologne, Germany
HPV Probe GP5+ modified	Operon, Cologne, Germany

Hydrochloric acid	Merck, Darmstadt, Germany
Isopropanol	Merck, Darmstadt, Germany
LE agarose	Seakem-BMA, ME, USA
<i>Mae</i> III restriction endonuclease	Roche Applied Science, Penzberg, Germany
Modified GP5+ probe	Operon, Cologne, Germany
Modified GP6+ reverse primer	Operon, Cologne, Germany
<i>Mse</i> I restriction endonuclease	New England Biolabs, USA
PGMY11-A forward primer	Operon, Cologne, Germany
PGMY11-B forward primer	Operon, Cologne, Germany
PGMY11-C forward primer	Operon, Cologne, Germany
PGMY11-D forward primer	Operon, Cologne, Germany
PGMY11-E forward primer	Operon, Cologne, Germany
Potassium chloride	Merck, Darmstadt, Germany
Potassium dihydrogen phosphate	Merck, Darmstadt, Germany
Proteinase K	Promega, USA
QIAprep Spin Miniprep Kit	Qiagen, Germany
<i>Rsa</i> I restriction endonuclease	New England Biolabs, USA
<i>Sfc</i> I restriction endonuclease	New England Biolabs, USA
Sodium chloride	Merck, Darmstadt, Germany
Sodium hydrogen phosphate	Merck, Darmstadt, Germany
Sodium hydroxide	Merck, Darmstadt, Germany
<i>Taq</i> DNA polymerase	New England Biolabs, USA
Tris base	Sigma, MO, USA
Triton X-100	Merck, Darmstadt, Germany
Tryptone	Difco Laboratories, MI, USA
Yeast extract	Gibco, NY, USA

APPENDIX B

INSTRUMENTS

Instrument-model	Source
Analytical balance	Sartorius, Germany
Autoclave	Kokusan, Japan
Biological safety cabinet class I	Esco, Singapore
DNA thermal Cycler	MJ Biorad, USA
Dry bath incubator	Major science, Taiwan
Horizontal gel electrophoresis system	BRL, USA
Hot Air Oven	Sheldon, USA
Low speed centrifuge 2500 RPM	Harikul, Thailand
Microcentrifuge, Denville 260D	Denville scientific, USA
Refrigerator (4°C)	Sanyo, Thailand
Four-Color Real-time PCR System, Chromo4™	Bio-Rad Laboratories, USA
Freezer (-20°C)	Sanyo, Thailand
Freezer (-70°C)	Kelvinator Scientific, USA
pH meter	Pierce, USA
Photo documentation	Fotodyne, USA
Primer Premier version 5.00 software	PREMIER Biosoft International,
Sero-fuge centrifuge	CA, USA
Spectrophotometer UV1201	Beckton Dickinson, USA
Spectrophotometer UV1201	Shimadzu Co., Japan
Ultraviolet transilluminator	Vilber Lourmat, France
Vortex mixer	Scientific Industries, USA
Water bath	Sheldon, USA

APPENDIX C

REAGENTS AND BUFFERS PREPARATION

1. Commonly used stock solutions

1 M Tris-HCl (pH 8.0)

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|-----------|---------|
| Tris base | 121.1 g |
|-----------|---------|
- Dissolve in 800 ml distilled water
 - Adjust the pH to 8.0 by adding concentrated HCl
 - Adjust the volume to 1 liter with distilled water
 - Sterilize by autoclaving and store at room temperature

0.5 M EDTA (pH 8.0)

- | | |
|---|---------|
| Disodium ethylenediaminetetra acetate·2H ₂ O | 186.1 g |
|---|---------|
- Dissolve in 800 ml distilled water
 - Adjust the pH to 8.0 by adding NaOH
 - Adjust the volume to 1 liter with distilled water
 - Sterilize by autoclaving and store at room temperature

2. Reagents for sample extraction

Phosphate buffer saline (PBS) pH 7.2

- | | |
|----------------------------------|--------|
| NaCl | 8 g |
| KCl | 0.2 g |
| Na ₂ HPO ₄ | 1.44 g |
| KH ₂ PO ₄ | 0.24 g |
- Dissolve in 800 ml distilled water

- Adjust the pH to 7.2 with HCl and fill up distilled water to 1 liter
- Sterilize by autoclaving and store at 4°C

Lysis buffer (50 mM Tris-HCl, 1mM EDTA, 1% Triton X-100)

1 M Tris-HCl pH 8.0	5	ml
0.5 M EDTA	0.2	ml
Triton X-100	1	ml

- Dissolve in 100 ml distilled water
- Sterilize by autoclaving and store at 4°C

3. Reagents for Electrophoresis

5X Tris-borate Buffer (TBE)

Tris base	54	g
Boric acid	27.5	g
0.5 M EDTA (pH 8.0)	20	ml

- Dissolve in 1 liter distilled water
- Sterilize by autoclaving and store at room temperature

0.5X Tris-borate Buffer (TBE)

Stock 5X TBE buffer	100	ml
Distilled water	900	ml

- Store at room temperature

6X gel loading buffer

Bromophenol blue	0.25	%
Glycerol	30	%

- Mix thoroughly and store at -20°C

10 mg/ml Ethidium bromide

Ethidium bromide	1 g
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- Dissolve in 100 ml distilled water
- Stir on a magnetic stirrer to ensure that the dye has dissolved
- Wrap the container in aluminum foil and store at room temperature

1% agarose gel

LE agarose	1 g
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0.5X TBE buffer	100 ml
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- Melt in microwave oven for 2-3 minutes

3% agarose gel

LE agarose	3 g
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0.5X TBE buffer	100 ml
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- Melt in microwave oven for 2-3 minutes twice or more until no seen the particle of agarose
- Boil in boiling water for 10 minutes for completely homogeneous of agarose

1 Kb Plus DNA Ladder

1 µg/µl 1 Kb Plus DNA Ladder™ (Invitrogen, USA)	5 µl
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6X gel loading buffer	10 µl
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Distilled water	45 µl
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- Mix thoroughly and store at -20°C

50 bp DNA Ladder

0.5 µg/µl 50 bp DNA Ladder GeneRuler™	10 µl
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(Fermentas, USA)

6X gel loading buffer	10 µl
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Distilled water	40 µl
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- Mix thoroughly and store at -20°C

4. Reagents for minipreparations of plasmid DNA

LB (Luria-Bertani) medium

Tryptone	10	g
Yeast extract	5	g
NaCl	5	g
- Dissolved in 800 ml distilled water		
- Adjust pH to 7.0 with NaOH and fill up distilled water to 1 liter		
- Sterilize by autoclaving and store at 4°C		

LB plates with ampicillin

Agar granulated	15	g
LB medium	1	l
- Sterilize by autoclaving and allow the medium to cool to 50°C		
- Add ampicillin to a final concentration of 100 µg/ml		
- Pour 30-35 ml of medium into 85 mm Petri dishes and let the agar harden		
- Store at 4°C for up to 1 month		

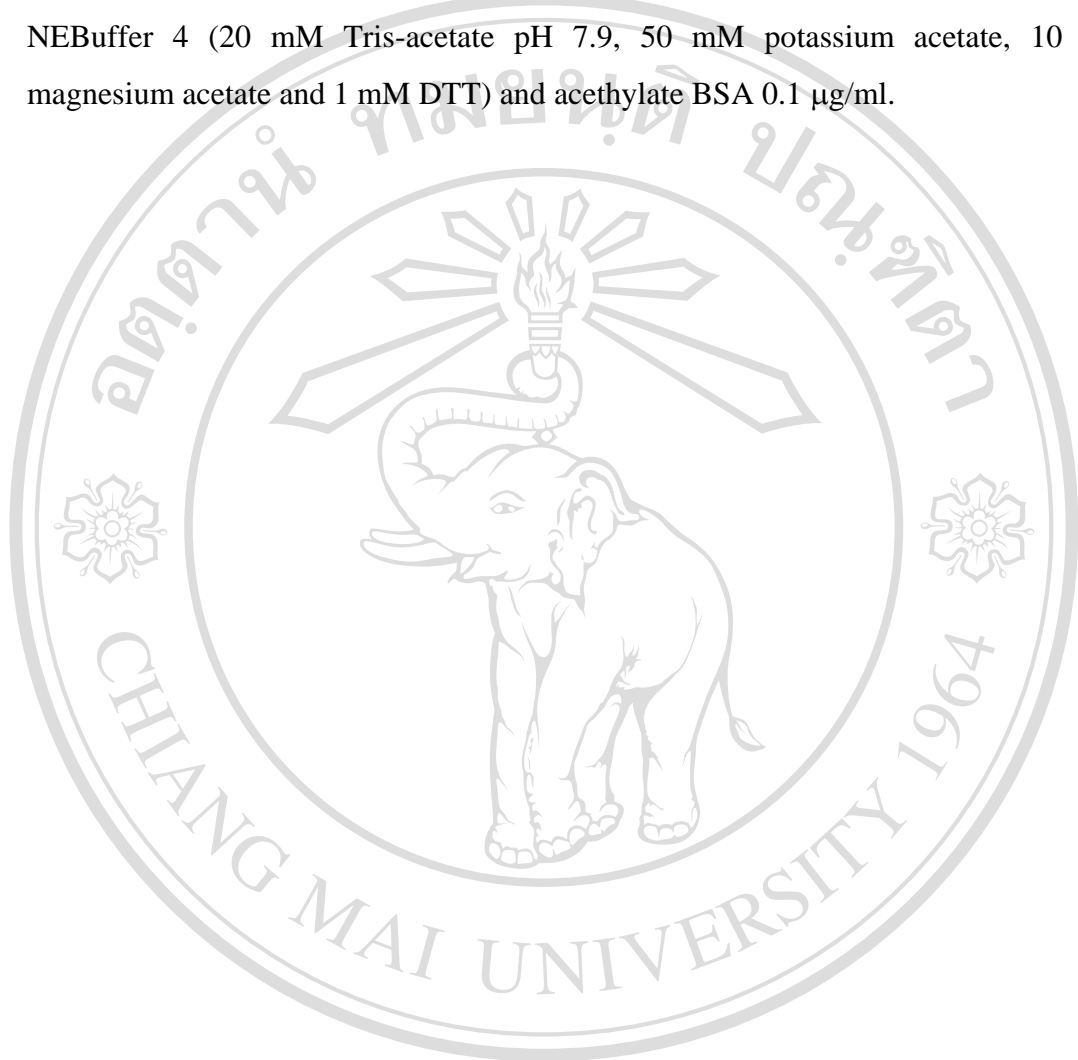
5. Reagents for Restriction Enzyme Analysis

MaeIII restriction endonuclease (Roche Applied Science, Penzberg, Germany) uses with 2x *MaeIII* incubation buffer (40 mM Tris-HCl pH 8.2, 550 mM NaCl, 12 mM MgCl₂ and 14 mM 2-Mercaptoethanol).

RsaI restriction endonuclease¹ (New England Biolabs, USA) uses with 10x NEBuffer 1 (10 mM Bis Tris Propane-HCl pH 7.0, 10 mM MgCl₂ and 1 mM dithiothreitol).

MseI restriction endonuclease (New England Biolabs, USA) uses with 10x NEBuffer 2 (10 mM Tris-HCl pH 7.9, 50 mM NaCl, 10 mM MgCl₂ and 1 mM DTT) and acetylate BSA 0.1 µg/ml.

SfcI restriction endonuclease (New England Biolabs, USA) uses with 10x NEBuffer 4 (20 mM Tris-acetate pH 7.9, 50 mM potassium acetate, 10 mM magnesium acetate and 1 mM DTT) and acethylate BSA 0.1 µg/ml.



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