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APPENDICES

APPENDIX A

List of chemicals and instruments

All chemicals used in the experiments were analytical grade. Names and sources of chemicals are listed below.

1.1 Chemicals

Chemicals	Sources
Absolute ethanol (C_2H_5OH)	Scharlau
Agarose gel electrophoresis	Bio-Rad Laboratories.
Ethidium bromide (C21H2ON3Br)	Sigma Chemical Co.
Ethylene diamine tetraacetic acid disodium Salt (EDTA) ($C_{10}H_{14}N_2Na_2O_8.2H_2O$)	Fluka chemika
Glacial acetic acid (CH_3COOH)	BDH Laboratory
Glycerol [$C_3H_5(OH)_3$]	Sigma Chemical Co.
Hydrochloric acid (HCl)	Merck
Isopropanol [$CH_3CH(OH)CH_3$]	BDH Laboratory
Sodium citrate ($Na_3C_6H_5O_72H_2O$)	BDH Laboratory
Sodium chloride (NaCl)	BDH Laboratory
Sodium dodecyl sulphate (SDS) ($Cl_6H_{25}NaO_4S$)	BDH Laboratory Supplies
Sodium hydroxide (NaOH)	BDH Laboratory Supplies
Sodium hydrogen carbonate ($NaHCO_3$)	Fluka chemika
Tris (hydroxyl aminomethane) ($C_4H_{11}NO_3$)	Gibco
Tris (hydroxylmethyl)-aminomethane hydrochloride ($C_4H_{11}NO_3HCl$)	Fluka BioChemika
Xylene	BDH Laboratory

1.2 Instruments

Instruments	Sources
Autoclave	Kokusan
Autopipette 0.5-10 µl	Jencons
Autopipette 5-50 µl	Jencons
Autopipette 50-200 µl	Jencons
Autopipette 100-1000 µl	Jencons
CO_2 incubator	Scientific Promotion

Gene Amp PCR System 2400	Perkin-Elmer
Hot air oven	WT binder
Lamina flow	Gelaire Laboratories
Incubator	Mammert
Microcentrifuge	New England Biolabs
Microwave	Turbora
pH meter	Fisher Scientific
Spectrophotometer	Ultraspec
Vortex mixer	Welp Scientific
Water bath	Struart Scientific

1.3 Enzymes and reagents

Enzymes and reagents	**Sources**
KpnI restriction endonuclease	New England BioLab
Hind III restriction endonuclease	New England BioLab
T4 DNA ligase	Invitrogen
dNTPs mixture	Invitrogen
DNA ladder 1 kp	Invitrogen
RNase A	Gentra system
Taq DNA polymerase	Fermentus
Proteinase K solution	Sigma Chemical Co.

APPENDIX B

Reagents for polymerase chain reaction

I. Reagents for polymerase chain reaction

1. 10XPCR buffer (Fermentus)
2. 25 mM $MgCl_2$ (Fermentus)
3. Taq polymerase 5 unit (Fermentus)

II. Reagents for agarose gel electrophoresis

1. Agarose gel preparation

	Agarose		0.5XTAE	
1.5% agarose	1.5	gm	100	ml
2.0% agarose	2.0	gm	100	ml

2. Loading dye

Bromophenol blue	0.125	gm
Glycerol	15	ml
10XTAE	30	ml

3. 10XTAE

Trisma base	48.4	gm
Glacial acetic acid	11.4	ml
0.5 EDTA pH 8.0	20	ml
Add H_2O to a final volume of	1,000	ml

4. 0.5 M EDTA (pH 8.0) 100 ml

EDTA	13.6	gm
Distilled water	100	ml

Adjust to pH 8.0 with 1M NaOH and autoclave 121°C, 15 lb, 15 min store at room temperature

5. Ethidium bromide (10 mg/ml)

Ethidium bromide	1	gm
Add H_2O to a final volume of	100	ml

6. DNA purification Kit

APPENDIX C

Reagents for reverse line blot hybridization assay

I. Reagent for reverse line blots hybridization

1. 2XSSPE 500 ml pH7.0

0.3M Sodium citate	44.1	gm
3M NaCl	876.5	gm
Distilled water	500	ml

2. 10% SDS

SDS	10	gm
Sterile water	100	ml

3. Chemiluminescent substrate

Substrate A: substrate B, 1:1

Substrate A	1	ml
Substrate B	1	ml

4. Developer solution 500 ml

Developer solution	125	ml
Distilled water	375	ml

5. Fixer solution 500 ml

Fixer solution A	100	ml
Fixer solution B	12.5	ml
Distilled water	387.5	ml

6. Streptavidin peroxidase conjugate

Streptavidin peroxidase conjugate	3	µl
1XPBS	12	ml

APPENDIX D

Reagents for construction of cloning vector and promoterless luciferase reporter vector

1. Reagents for competent cells preparation and plasmid transformation

1.1 LB broth 1,000 ml

Typtone	10	gm
Yeast extract	5	gm
NaCl	10	gm
Distilled water	1,000	ml

Autoclave 121°C, 15 lb, 15 min, store at 4°C

1.2 LB agar

Typtone	10	gm
Yeast extract	5	gm
NaCl	10	gm
Bacto-agar 1.5%	15	gm
Distilled water	1,000	ml

1.3 0.1 M $CaCl_2$ 100 ml

$CaCl_2$	1.47	gm
Distilled water	100	ml

Autoclave 121°C, 15 lb, 15 min, store at 4°C

1.4 50 mM $CaCl_2$ 100 ml

0.1 M $CaCl_2$	50	ml
Distilled water	50	ml

Autoclave 121°C, 15 lb, 15 min, store at 4°C

1.5 50 mM $CaCl_2$+15%glycerol 100 ml

50 mM $CaCl_2$	62.5	ml
40%glycerol	37.5	ml

Autoclave 121°C, 15 lb, 15 min, store at 4°C

1.6 Amplicillin 200 mg/ml

Amplicillin	1	gm
Distilled water	5	ml

Aliquot 200 ul/tube and stored at –70°C until used (working= 50 ug/ml)

1.7 Purelink™ Quick Plasmid Miniperp Kit (Invitrogen)

APPENDIX E
CELL LINE CULTURE

1. 1X DMEM (Dulbecco-MEM;Gibco-BRL,Gaithersburg,MD)

2. 10%Fetal Bovine Serum (FBS;Seromed, Berlin,Germany)

3. Antibiotics stock

3.1 Penicillin 200,000 units/ml (100,000 units/ aliquot)

Penicillin	1,000,000	units
Sterile water	5	ml
Aliquot 500 ul/tube and use 500 ul to media	1,000	ml
(working= 100 units/ml)		

3.2 Streptomycin 200,000 ug/ml (1000,000 ug/ aliquot)

Streptomycin	1	gm
Sterile water	5	ml
Aliquot 500 ul/tube and use 500 ul to media	1,000	ml
(working= 100 ug/ml)		

3.3 Gentamicin (40 mg/aliquot)

Gentamicin 80 mg/2 ml	1	ampule
Aliquot 1 ml/tube and use 1 tube to media	1,000	ml
(working= 40 mg/ml)		

3.4 Fungizone 5,000 ug/ml (2,500 ug/ aliquot)

Fungizone (1 vial)	50	mg
Sterile water	10	ml
Aliquot 500 ul/tube and use 500 ul to media	1,000	ml
(working= 2.5 ug/ml)		

Don't forget protection of fungizone will be labile with cover tube

4. Phosphate buffered saline (PBS) (1X) pH 7.5

NaCl	8.00	gm
KCl	0.20	gm
KH_2PO_4	0.12	gm
Na_2HPO_4 (anhydruos)	0.91	gm
Deionized distilled water to	1,000	ml

Autoclave 121°C, 15 lb, 15 min and store at 4°C

5. 0.25 % Trypsin-EDTA 1:5,000

Trypsin powder (1:300)	0.25	gm
EDTA	0.02	gm
PBS 1X pH 7.5	100.00	ml

The solution is passed through ash-free filter paper, then sterile by Millipore filtration and store at 4°C

6. DMEM (1X)

DMEM powder		
Deionized distilled water	1,000	ml
$NaHCO_3$	3.7	gm

The solution is passed through filter paper, then sterile by Millipore filtration and store at 4°C

7. 10%FCS+DMEM(1X) 100 ml

Opti-MEM medium solution	90	ml
Fetal calf serum (FCS)	10	ml

RESEARCH PRESENTATION

Parichat Wongwarissara, Tipaya Ekalaksananan, Chamsai Pienthong, Kobkul Tungsinmunkong, Cheepsumon Suthippintawong, Songkhun Vinyuvat (2009, February 12-13). Analysis of HPV16 Long Control Region (LCR) polymorphism in Squamous Cell Cervical Carcinoma. The 10th Graduate Research Conference, Khon Kaen University 2010, Khon Kaen, Thailand. (Oral presentation).

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