IX. APPENDIXES

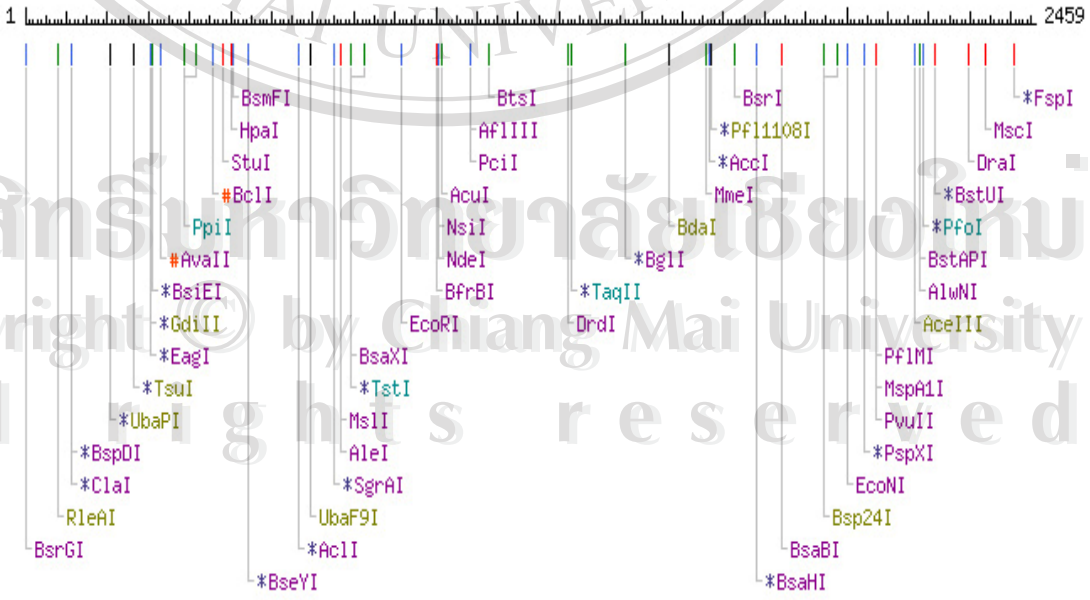
APPENDIX A

Restriction Map of *hsp70* gene of *P. marneffei*

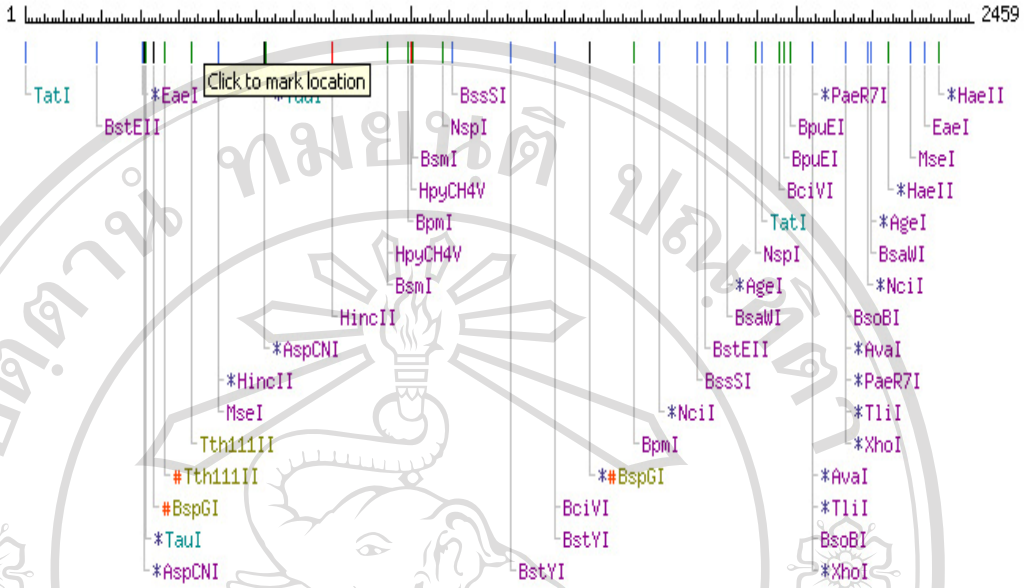
A.) List of zero cutter

*Aar*I, *Aat*II , *Afe*I, *Afl*II, *Ahd*I, *Aju*I, *Alf*I, *Alo*I, *Alw*FI, *Apa*I, *Apa*LI, *Asc*I, *Ase*I, *Asi*SI, *Avr*II, *Bae*I, *Bam*HI, *Bfa*I, *Bfu*AI, *Bgl*II, *Bme*1580I, *Bmg*BI, *Bmg*I, *Bmr*I, *Bmt*I, *Bsa*AI , *Bsg*I, *Bsi*WI, *Bsp*EI, *Bsp*MI, *Bsr*BI, *Bss*HII, *Bss*IMI, *Bst*BI, *Bst*XI, *Bst*Z17I, *Btg*ZI, *Csp*CI, *Dra*III, *Eco*O109I, *Eco*RV, *Fal*I, *Fse*I, *Fsp*AI, *Hae*IV, *Hgi*EII, *Hin*dIII, *Kas*I, *Mfe*I, *Mlu*I, *Nae*I, *Nar*I, *Ngo*MIV, *Nhe*I, *Not*I, *Nru*I, *Pac*I, *Pas*I, *Pfl*FI, *Pme*I, *Pml*I, *Ppu*MI, *Psh*AI, *Psi*I, *Psp*OMI, *Psr*I, *Pst*I, *Pvu*I, *Rsr*II, *Sac*II, *Sal*I, *San*DI, *Sap*I, *Sbf*I, *Sca*I, *Sex*AI, *Sfc*I, *Sfi*I, *Sfo*I, *Sma*I, *Sma*II, *Spe*I, *Sph*I, *Srf*I, *Sse*232I, *Sse*8647I, *Ssp*I, *Swa*I, *Tso*I, *Tth*111I, *Uba*F7I, *Xba*I, *Xcm*I, *Xma*I, *Xmn*I, *Zra*I

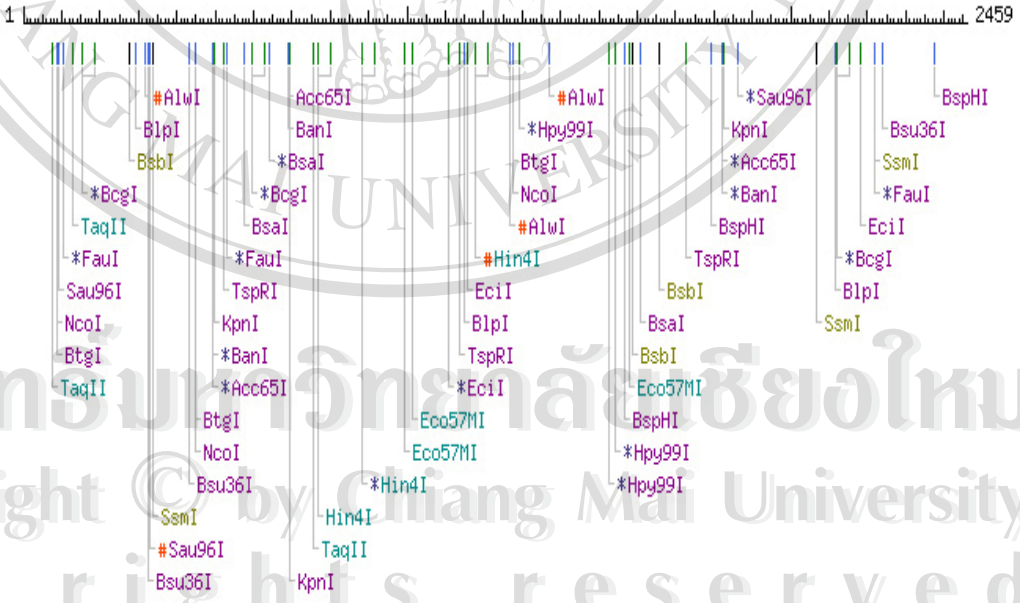
B.) Graphical map of one cutter



C.) Graphical map of two cutter



D.) Graphical map of triple cutter



APPENDIX B

Recipes

A. Culture Media

1) Brain heart infusion (BHI) agar

BHI agar (dehydrated)	52.00	g
Distilled water	1,000.00	ml

Melt, disperse in tubes and autoclave at 121 °C 15 lbs for 15 min. Allow tubes to cool in slant position. Or, after autoclave, pour plates and allow to cool.

2.) BHI broth

BHI	37.00	g
Distilled water	1,000.00	ml

Dissolve, disperse 30 ml in each 125 ml-Erlenmeyer flask. Autoclave at 121 °C 15 lbs for 15 min.

3.) Luria-Bertani (LB) Medium

LB	20.00	g
Deionized H_2O up to	1,000.00	ml

Shake until the solutes are dissolved. Sterilize by autoclaving for 20 min at 15 lbs on liquid cycle.

4.) Sabouraud Dextrose Agar (SDA)

Dehydrated SDA agar (Becton Dickinson)	65.00	g
Distilled water	1,000.00	ml

Suspend 65 g of the powder in 1 liter of distilled water. Mix thoroughly, heat with frequent agitation and boil for 1 min to completely dissolve the powder. Autoclave at 121 °C 15 lbs. for 15 min.

5.) SOC medium

Per liter:

To 950 ml of deionized water, add:

Tryptone	20	g
Yeast extract	5	g
NaCl	0.5	g

Shake until the solutes are dissolved. Adjust the pH to 7.0 with 5 N NaOH. Adjust the volume of the solution to 980 ml with the deionized water. Sterile by autoclaving, allow it to cool to 60 ^{o}C or less and then add 20 ml of a sterile 1 M solution of glucose.

The glucose solution is made by dissolving 18 g of glucose in 90 ml of deionized water. After the sugar is dissolved, adjust the volume of the solution to 100 ml and sterile by filtration through a 0.2-μm filter.

B.) Solutions for working with bacteriophage λ

1.) 20 % Maltose

Maltose	20	g
H_2O to	100	ml

Sterilize the solution by filtration through a 0.2-μM filter. Store the sterile solution at room temperature.

2.) SM

Per liter:

NaCl	5.8	g
$MgSO_4 \cdot 7H_2O$	2	g
1 M Tris-Cl (pH 7.5)	50	ml
2 % gelatin solution	5	ml
H_2O to	1	L

Sterilize the buffer by autoclaving for 20 min ar 15 lb/sq.in. on liquid cycle. After the solution is cooled, dispense 50-ml aliquots into sterile containers. SM may be stored indefinitely at room temperature.

A 2 % gelatin solution is made by adding 2 g of gelatin to a total volume of 100 ml of H_2O and autoclaving the solution for 15 min at 15 lb/sq.in. on liquid cycle.

3.) 10 mM $MgSO_4$ (λ diluent)

Per liter:

1 M Tris-Cl (pH 7.5)	10	ml
$MgSO_4 \cdot 7H_2O$	2	g
H_2O to	1	L

Sterilize the buffer by autoclaving for 20 min at 15 lb/sq.in. on liquid cycle. After the dilution has cooled, dispense 50-ml aliquots into sterile containers. λ diluent may be stored indefinitely at room temperature.

C.) Alkaline lysis buffers for minipreparation of plasmid DNA

1.) Solution I

50 mM glucose
25 mM Tris-Cl (pH 8.0)
10 mM EDTA (pH 8.0)

Sterilize by autoclaving for 20 min at 15 lb/sq.in. on liquid cycle and store at 4°C.

2.) Solution II

0.2 N NaOH (freshly diluted from a 10 N stock)
1 % SDS

3.) Solution III

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.

D.) Solutions for working in genomic DNA extraction

1.) Osmotic medium [pH 7.0]

- 0.6 M sorbitol
- 100 mM Tris-HCl

Sterilize by autoclaving for 20 min at 15 lb/sq.in. on liquid cycle and store at 4^{o}C.

2.) ST buffer [pH 5.8]

- 1.2 M MgSO4
- 10 mM sodium phosphate

Sterilize by autoclaving for 20 min at 15 lb/sq.in. on liquid cycle and store at 4^{o}C.

3.) Lysis buffer [pH 8.0]

- 10 mM Tris
- 1 mM EDTA
- 1% sodium dodecyl sulphate

Sterilize by autoclaving for 20 min at 15 lb/sq.in. on liquid cycle and store at 4^{o}C.

E.) Reagents

1.) 50X Tris-acetate (TAE) buffer

Tris base	242	g
Glacial acetic acid	57.1	ml
0.5 M EDTA	100	ml

The working solution (1X) contains 0.04 M Tris acetate and 0.0001 M EDTA

2.) 6X agarose gel-loading buffer

Bromophenol blue	0.025	g
Xylene cyanol FF	0.025	g
Glycerol	3	ml
Distilled water	6.95	ml

X. CURRICULUM VITAE

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