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Appendices

ศูนย์วิทยทรัพยากร  
จุฬาลงกรณ์มหาวิทยาลัย

## Appendix A

## The reagents for experiments

1. virus isolation

## Trypsin versience

- Stock Trypsin (2.5% trypsin)	5	ml
- Stock versience (1% EDTA)	2.5	ml
- PBS	92.5	ml

2. Indirect immunoperoxidase monolayer assay (IPMA)

## 0.5%PBST

- 20xPBS	80	ml
- DW	1,920	ml
- Tween20	10	ml

## 4% formalin in 0.5%PBST

- 40% formalin	0.4	ml
- 0.5%PBST	9.6	ml

## 1% BSA in 0.5% PBST

- BSA	0.15	g
- 0.5%PBST	15	ml



The SDOW-17 (1:300)

- SDOW-17	20	μl
- 1% BSA in 0.5% PBST	6	ml

The mouse IgG conjugate (1:300)

- The mouse IgG conjugate	20	μl
- 1% BSA in 0.5% PBST	6	ml

substrate for IPMA

- AEC solution	0.5	ml
- acetate buffer	9.5	ml
- 30% H <sub>2</sub> O <sub>2</sub>	25	μl

AEC solution

- 3-amino acid-9-ethylcarbazole	80	mg
- Dimethyl formamide	20	ml

Acetate buffer

- 0.1 M Glacial acetic acid	21	ml
- 0.1 M Sodium acetate	79	ml

### 3. RNA extraction

QIAamp<sup>®</sup> Viral RNA Mini Kit. (QIAquick ion-exchange column chromatography)

- Buffer AVL; containing carrier RNA
- AW1 and AW2; two different wash buffers, has significant improved the purity of the eluted RNA

- AVE; RNase-free water that contains 0.04% sodium azide to prevent microbial growth and subsequent contamination with RNases.

#### 4. Reverse transcriptase polymerase chain reaction

QIAGEN<sup>®</sup> One Step RT-PCR Kit , USA)

- QIAGEN One step RT-PCR Enzyme Mix; Omniscript Reverse Transcriptase, Sensiscript Reverse Transcriptase and HotStar Tag DNA polymerase. Omniscript and Sensiscript Reverse Transcriptase are recombinant heterodimeric enzymes expressed in *E. coli*. HotStar Tag DNA polymerase is a modified form of a recombinant 94-kDa DNA polymerase (deoxynucleoside-triphosphate : DNA deoxynucleotidyltransferase, EC 2.7.7.7), originally isolated from *Thermus aquaticus*, expressed in *E. coli*.
- 5xQ-solution; 12.5 mM MgCl<sub>2</sub>
- 5xQIAGEN One step RT-PCR Buffer; KCl and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>

2% agarose gel

- agarose gel 0.1 g
- TBE (Tris-borate/EDTA) 50 ml

#### 5. PCR product purification

The QIAquick<sup>®</sup> spin

- Buffer PB; guanidine hydrochloride and isopropanol
- Buffer PE; ethanol containing buffer PE

6. Restriction fragment length polymorphism

Buffer R<sup>+</sup>; 10mM Tris-HCl (pH 8.5), 10mM MgCl<sub>2</sub>, 100mM KCl, 0.1 mg/ml BSA

Buffer 2xγ<sup>+</sup> TANGO™; 33mM Tris-acetate, 10mM magnesium acetate, 66mM potassium acetate, 0.1 mg/ml BSA (pH 7.9 at 37<sup>o</sup>c)

2x γ<sup>+</sup> TANGO™

- 10x γ<sup>+</sup> TANGO™ 10 μl

- BSA 40 μl

1x γ<sup>+</sup> TANGO™

- 10x γ<sup>+</sup> TANGO™ 5 μl

- BSA 45 μl

Buffer B<sup>+</sup>; 10mM Tris-HCl (pH 7.5), 10mM MgCl<sub>2</sub>, 0.1 mg/ml BSA

1x NEBuffer3

- 10x NEBuffer3 5 μl

- 1x BSA 45 μl

7. Immunohistochemistry

DAB solution

- 05% Diaminobenzidinetetrachloride 0.075 g

- 30% H<sub>2</sub>O<sub>2</sub> 50 μl

- Tris buffer 150 ml

## Appendix B

IDEXX<sup>®</sup> ELISA titers to PRRSV and viral titers from PRRSV-infected pigs in 0, 5, 9 and 15 day post inoculation.

dpi.	0		5		9		15	
No.	Ab(S/P) <sup>a</sup>	PRRSV <sup>b</sup>	Ab(S/P) <sup>a</sup>	PRRSV <sup>b</sup>	Ab(S/P) <sup>a</sup>	PRRSV <sup>b</sup>	Ab(S/P) <sup>a</sup>	PRRSV <sup>b</sup>
EU/1	0.055(-)	0	0.071(-)	10 <sup>2</sup>	0.847(+)	10 <sup>1.5</sup>	-	-
EU/2	0.017(-)	0	0.034(-)	10 <sup>2.75</sup>	-	-	-	-
EU/3	0.122(-)	0	0.055(-)	10 <sup>2</sup>	0.905(+)	10 <sup>2.75</sup>	-	-
EU/4	0.088(-)	0	-	-	-	-	-	-
EU/5	0.057(-)	0	0.011(-)	10 <sup>2.33</sup>	0.943(+)	10 <sup>1.5</sup>	0.814(+)	10 <sup>1.5</sup>
EU/6	0.031(-)	0	0.055(-)	10 <sup>3.25</sup>	-	-	-	-
EU/7	0.149(-)	0	0.052(-)	10 <sup>1</sup>	1.074(+)	10 <sup>2.75</sup>	-	-
EU/8	0.132(-)	0	0.168(-)	10 <sup>2.75</sup>	0.079(-)	10 <sup>1</sup>	0.632(+)	10 <sup>2.0</sup>
EU/9	0.046(-)	0	0.105(-)	10 <sup>1.75</sup>	1.409(+)	10 <sup>2</sup>	0.198(-)	10 <sup>1.5</sup>
US/1	0.046(-)	0	0.204(-)	10 <sup>2.5</sup>	-	-	-	-
US/2	0.021(-)	0	0.071(-)	10 <sup>2.5</sup>	-	-	-	-
US/3	0.044(-)	0	0.010(-)	10 <sup>2.66</sup>	1.016(+)	10 <sup>2.5</sup>	-	-
US/4	0.015(-)	0	0.097(-)	10 <sup>3</sup>	0.404(+)	10 <sup>3</sup>	-	-
US/5	0.027(-)	0	0.082(-)	10 <sup>2.5</sup>	-	-	-	-
US/6	0.025(-)	0	0.036(-)	10 <sup>2.3</sup>	0.905(+)	10 <sup>3.5</sup>	-	-
US/7	0.094(-)	0	0.233(-)	10 <sup>2.5</sup>	0.691(+)	10 <sup>2.75</sup>	0.681(+)	10 <sup>0.25</sup>
US/8	0.090(-)	0	0.013(-)	10 <sup>2.5</sup>	1.266(+)	10 <sup>3.25</sup>	-	-
US/9	0.032(-)	0	0.092(-)	10 <sup>2.5</sup>	0.677(+)	10 <sup>2.75</sup>	0.866(+)	10 <sup>1.5</sup>
Con/1	0.078(-)	0	0.141(-)	0	-	-	-	-
Con/2	0.002(-)	0	0.008(-)	0	0.033(-)	0	-	-
Con/3	0.034(-)	0	0.048(-)	0	0.000(-)	0	0.009(-)	0

<sup>a</sup> S/P  $\geq$  0.4 positive, S/P < 0.4 negative (S/P; OD sample / OD positive control positive control (OD) = 0.524 negative control (OD) = 0.094)

<sup>b</sup> PRRS virus isolation (TCID<sub>50</sub>/50  $\mu$ l)



The summary gross lesions in other organs of 5, 9, 15 dpi. inoculated pigs.

No.	Gross lesions
EU/2	Petechial hemorrhage of renal cortex
EU/6	Lymphadenopathy, inguinal l.n. (2x), Petechial hemorrhage of renal cortex
US/1	Lymphadenopathy, inguinal l.n. (2x)
US/2	Lymphadenopathy, inguinal l.n. (2x), mesenteric l.n. (3x), diphtheritic tonsillitis
US/5	Lymphadenopathy, inguinal l.n. (2x), Petechial hemorrhage of both renal cortex, yellowish liver
Con/1	-
EU/1	Lymphadenopathy, inguinal l.n. (2x)
EU/3	Lymphadenopathy, inguinal l.n. (2x)
EU/7	Lymphadenopathy, inguinal l.n. (2x)
US/3	Lymphadenopathy, inguinal l.n. (2x), tracheobronchial l.n.
US/6	Lymphadenopathy, inguinal l.n. (2x), tracheobronchial l.n (2x), pleuritis
US/8	Lymphadenopathy, inguinal l.n. (3x)
Con/2	-
EU/5	Lymphadenopathy, inguinal l.n. (3x), tracheobronchial l.n (3x)
EU/8	Lymphadenopathy, inguinal l.n. (4x), tracheobronchial l.n (3x)
EU/9	Lymphadenopathy, inguinal l.n. (2x), tracheobronchial l.n (2x), multiple white foci in renal cortex
US/4	Dead, Lymphadenopathy, inguinal l.n. (4x), tracheobronchial l.n (4x), fibrinous pleuritis, peritonitis pericarditis, splenomegaly
US/7	Lymphadenopathy, inguinal l.n. (3x), mild enteritis
US/9	Lymphadenopathy, inguinal l.n. (2x), tracheobronchial l.n (2x), focal petechial hemorrhage of right kidney
Con/3	Lymphadenopathy, inguinal l.n. (1.5x)

Neutralizing Peroxidase Link Assay (NPLA) from PRRSV-infected pigs in 0, 5, 9 and 15 day post inoculation.

dpi.	0	5	9	15
No.	NPLA titer			
EU/1	16	8	8	-
EU/2	32	16	-	-
EU/3	2	4	8	-
EU/4	64	-	-	-
EU/5	16	16	8	8
EU/6	16	8	-	8
EU/7	16	8	4	-
EU/8	4	4	4	-
EU/9	32	32	16	16
US/1	4	4	-	-
US/2	8	8	-	-
US/3	16	4	8	-
US/4	8	4	4	-
US/5	16	16	-	-
US/6	16	4	16	-
US/7	64	32	16	8
US/8	64	4	16	-
US/9	16	16	16	16
Con/1	64	32	-	-
Con/2	8	8	8	-
Con/3	16	8	8	2

## 02SP2 [US genotype]

### 464 base pairs

gtgccgttctgtttgtgctgcgctcgccaacgccagcaacagcagctcccacttacagttgattataacctgacg base pairs  
cacggcaagacaaaacgacgcgagcgggtgcggtcgtgtcgcgagggggaatgtcaactaaatattggactgc 1 to 75

atatgtgagctgaatggcactgattggctgaaagacaattttgattgggcagtgagactttgtcatctttccc base pairs  
tatacactcgacttaccgtgactaaccgactttctgttaaaactaaccgctcacctctgaaaacagtagaaaggg 76 to 150

gtattgactcatatagctcctacggtgccctcaccaccagccattttctgacgcagttggtctaactactgtg base pairs  
cataactgagtatacagaggatgccacgggagtggtgctgtaaaagaactgcgtcaaccagattagtgacac 151 to 225

tccaccgccgatattaccatggcggtatgtcttgagcagtatctacgctgtctgtgccctggctgcgctaatt base pairs  
aggtggcggcctataatggtaccgcatacagaactcgtcatagatgcgacagacacgggaccgacgcgattaa 226 to 300

*HincII*

tgctttattattaggttgacaagaactgtatgtcctggcgtattcatgtaccagatataccaactttgtctg base pairs  
acgaaataataatccaactgtttcttgacatacaggaccgcgataagtacatggtctatatggttgaacaagac 301 to 375

*HaeIII*

gacaccaaaagccagctctatcgttgccggtgcccgctcatcatagagaaggggggtaaagctgaagttggaggt base pairs  
ctgtggtttccggtcgagatagcaaccgccagcgggcagtagtatcttccccccatttcgacttcaacctcca 376 to 450

cacctgatcgacct base pairs  
gtggactagctgga 451 to 464

**Table by Enzyme Name**

Enzyme name	No. Positions cuts of sites	Recognition sequence	
<i>HaeIII</i>	1 386	gg/cc	<a href="#">More info</a>
<i>HincII</i>	1 317	gty/rac	<a href="#">More info</a>

The following endonucleases were selected but don't cut this sequence:  
*MluI, SacII*

## 01NP2 [US genotype]

### 505 base pairs

*SacII*

ggtggggaatgcttgaacgcgggctcctgctcgcgattgcctttttgtggtgatcgtgccgttctgttttct base pairs  
caacccttacgaactggcggccgaggacgagcgcctaacggaacacccacatagcacggcaagacaaaacga 1 to 75

gcgctcgccaacgccagcaacagcagctcccacttacagttgattataacctgacgatatgtgagctgaatgcc base pairs  
cgcgagcgggtgcggtcgttcgctcgagggtgaatgtcaactaaatattggactgctatacctcgcactaccg 76 to 150

actgattggctgaaacataatattgattggcagtgagactttgtcatcttcccgtactgactcatatagtc base pairs  
tgactaacggactttgtattaaaactaacccgtcacctctgaaaacagtagaaggggcatgactgagtatatcag 151 to 225

tcctacggtgccctcaccaccagccattttctgacgcagttggtctaactcactgtgtaccgccgatattac base pairs  
aggatgccacgggagtggtggtcgtaaaagaactcgcgcaaccagattagtgacacagatggcggcctataatg 226 to 300

*HincII*

catggcggtatgtcttgagcagtatctacgctgtctgtgccctagctgcgctaattgctttattagggtg base pairs  
gtaccgccatacagaactcgtcatagatgctgacagacacgggatcgcgcataaacgaaataataatccaac 301 to 375

acaaagaactgtatgctcctggcgttattcatgtaccagatataccaacttgttctggacaccaagggcaagctc base pairs  
tgttcttgacatacagaccgcaataagtacatggtctatatggttgaacaagacctggttcccgttcgag 376 to 450

tatcgttggcggctgccgctcatcatagagaaaggggtaaagttgaagttggag base pairs  
atagcaaccgccagcggcagtagtatctttcccccattcaactcaacctc 451 to 505

**Table by Enzyme Name**

Enzyme name	No. Positions cuts of sites	Recognition sequence	
<i>HincII</i>	1 374	gty/rac	<a href="#">More info</a>
<i>SacII</i>	1 20	ccgc/gg	<a href="#">More info</a>

The following endonucleases were selected but don't cut this sequence:  
*HaeIII*, *MluI*

## 02PB1 [US genotype]

### 442 base pairs

cgcttttttggtacatcgctgccgttctgttttctgctgctcagcgcagcaacggcagcagctcccact base pairs  
gcggaaaaaacaccatgtagcacggcaagacaaaacgacgcgagcagtcgctgctgcccgcgaggggtga 1 to 75

tacagttgatctataacctgacgatatgtgagctgaacggcacagagtggtgaatgaaagtttgattgggcag base pairs  
atgtcaactagatattggactgctatacactgacttgcctgtctcaccgacttactttcaaaactaacccgtc 76 to 150

*HincII*

tggaaaccttgtcattttcccggttgacacatagctctctacggcgcctcaccaccagtcacttcttg base pairs  
accttggaaacagtaaaaagggcacaactgagtatatcagaggatgccgaggagtggtggtcagtaaggaac 151 to 225

atacagtcggtctgaccacgtggctaccgcccgggtattaccatgggcggtatgtcttgagcagcatctacgccg base pairs  
tatgtcagccagactggtggcaccgatggcgcgccataatggtaccgccatacagaactcgtcgtagatgccc 226 to 300

tatgtccttagctgcgctgatttgctcatcattagttggcaagaactgtatgtctggcgctattcatgta base pairs  
atcacggaatcgacgcgactaaacgaagtagtaatccaaccgttcttgacatacaggaccgcgataagtagat 301 to 375

ccagatataccaacttcttctggacaccaaaggcaagctctatcgttggcgatcgctgtcatcat base pairs  
ggtctatagttgaaagtaagacctgtggttccgttcgagatagcaaccgtagcggacagtagta 376 to 442

#### Table by Enzyme Name

Enzyme name	No. Positions cuts of sites	Recognition sequence	More info
<i>HincII</i>	1 178	gty/rac	<a href="#">More info</a>



## US-MLV vaccine 603 base pairs

*SacII*

atgttgagaaatgctgacccgctgtgctcgcattgctttttgtggtatcgtgccgttctgttt base pairs  
tacaacctctttacgaactggcgcccgacaacgagcgttaacgaaagaacaccacatagcacggcaagacaaaa 1 to 75

gctgtctcgaacgccagcaacgacagcagctcccctctacagctgattacaactgacgctatgtgagctg base pairs  
cgacacgagcgggtgcggctgtgctgctcgaaggtagatgctgactaaatgtgaactgcgatacactcgac 76 to 150

aatggcacagattggctagctaacaatttgattgggcagtgagagtttgcctcttcccgtttgactcac base pairs  
ttaccgtgtctaaccgatcgattgttaactaacccgtcacctcctcaaacagtagaaaggcgaactgagtg 151 to 225

attgtcctatggtgccctcactaccagccatttcttgacacagtcgctttagtcaactgtgtctaccgccggg base pairs  
taacagaggataccacgggagtgatggtcggtaaaaggaaactgtgtcagcgaatcagtgacacagatggcgccc 226 to 300

*HincII*

ttgttcacggcggtatgtcctaagtagcatctacggctgtgcccctggctgcttgcatt base pairs  
aaacaagtgcccgccatacaggattcatcgtatgctccagacacgggaccgacgcaactgaacgaagcagtaa 301 to 375

*MluI*

aggttgcaagaattgcatgtcctggcgtacggctgtaccagatataccaacttcttggacactaagggc base pairs  
tccaaactttcttaactacagagccgcatgctcacatggtctatatggtgaaagaagacctgtgatcccg 376 to 450

ggactctatcgtggcggtgcctgtcatcatagagaaaaagggcaagttgaggtcgaaggtcatctgatcgac base pairs  
cctgagatagcaaccgccagcgacagtagtatctctttccccgtttcaactccagcttccagtagactagctg 451 to 525

ctcaaaagagttgtgcttgatgggtccgtggcaaccctgtaaccagagtttcagcggacaatggggtcgtcct base pairs  
 gagttttctcaacacgaactaccaaggcaccgtggggacattggtctcaaagtcgcttgttaccacagagga 526 to 600

tag base pairs  
 atc 601 to 603

#### Table by Enzyme Name

Enzyme name	No. cuts of sites	Positions	Recognition sequence	More info
<i>HincII</i>	1	359	gtv/rac	<a href="#">More info</a>
<i>MluI</i>	1	407	a/cgct	<a href="#">More info</a>
<i>SacII</i>	1	23	ccgc/gg	<a href="#">More info</a>

The following endonucleases were selected but don't cut this sequence:  
*HaeIII*

ศูนย์วิทยทรัพยากร  
 จุฬาลงกรณ์มหาวิทยาลัย

## 01CB1 [EU genotype]

### 479 base pairs

ctcgactaccaatacatatataatttgacgatatgagctgaatgggaccgctggctgccaccattttat base pairs  
gagctgatggttatgtatatataaactgctatacgtcgtactaccctggcggaccgacaggctgtaaaaaa 1 to 75

tgggcagctgagactttgtgctctaccagtgggactcacattctcactgggttcctacgacaagccac base pairs  
accctcagctctgaaaacacgagatgggtcaccgtgagtgtaagagagtgacccaaaggaatgctgttcggtg 76 to 150

ttccttgacgcgctcggcctcggagctgtgtctgttacaggattccatggcggcggtactcagcagcgtg base pairs  
aaggaaactgcgagccggagcctcgacacagacaatgtcctaaggtaccgcccctatgcatgagctgctgcac 151 to 225

*Hae*II *Hae*II  
tacggcgtgtgctgctagcagccttgcacgtttgtcatccgtgctgtcaaaaattgtatggcttggcgtat base pairs  
atgccggaacacgcgatcgtcgcgagcgtacgaaacagtaggcacgacagttttaacataccgaacggcgata 226 to 300

gctcgtaccgggtcaccaacttcattgtggacgaccgtgggagaatccatcggtggaggtctccaatagtggtg base pairs  
cgagcatggccaagtgtgtaagtaaacctgctggcaccctcttaggtagccacctccagaggtatcaccac 301 to 375

gaaaaattgggtaaagctgtgtcggcagcaaccttgcaccatcaaacatgtttctcgaaggagtaaaagct base pairs  
cttttaaccatttcgacaacagccgtcgttgaacagtggtagttgtacaacaagagcttctcattttcga 376 to 450

caacccttgacgaggacttcgcccagca base pairs  
gttgggaactgctcctgaagccggctcgt 451 to 479

**Table by Enzyme Name**

Enzyme name	No. Positions cuts of sites	Recognition sequence	More info
<i>Hae</i> II	2 233 250	rgcg/y	<a href="#">More info</a>

The following endonucleases were selected but don't cut this sequence:  
*Cl*I, *P*stI

## 02RB1 [EU genotype]

### 498 base pairs

ggcaacggcaacagctcgacataccaatacatatataacctgacgatgagctgaatgggaccgctggctg base pairs  
ccgttgccgtgtgagctgtatggtatgtatatattggactgctatacgctcgactaccctggcggaccgac 1 to 75

tccgaccattttattggcagctgagactttgtgctctaccagtgccgactcacattctcactgggttc base pairs  
aggctggtaaaaataaccctgacgctctgaaaacacgagatgggtcaccgctgagtgtaagagagtgacccaaag 76 to 150

cttacgacaagccacttcttgacgcgctcgccctcgagctgtgtctgttacaggattccatggcgggctgtac base pairs  
gaatgctgtccggtgaaggaactgcgcgagccggagcctcgacacagacaatgtcctaaggtaccgcccccatg 151 to 225

gtactcagcagcgtgtacggcctgtgcgctagcagcgcctgcgcatgctttgtcatcctgctgtcaaaaattgt base pairs  
catgagtcgtcgacatgcccgaacacgcatcgtcgcgagcgtacgaaacagtaggcacgacagttttaaca 226 to 300

atggcttggcctatgctcgtaccgggtcaccaactcattgtggagcaccgtgggagaatccatcggtggagg base pairs  
taccgaacggcgatacagcagcatggccaagtgtggaagtaacacctgctggcaccctctaggtagccacctcc 301 to 375

tctccaatagtggtgaaaaattgggtaaaagctgtgctggcagcaacctgtcaccatcaaacatgttttctc base pairs  
agaggtatcaccaccttttaaccatttcgacaacagccgtcgttggaaacagtggtagttgtacaacaagag 376 to 450

gaaggagtaaaagctcaaccctgacgagacttcggccgagcaatgg base pairs  
cttctcattttcgagttgggaactgctctgaagccggctgtacc 451 to 498

#### Table by Enzyme Name

Enzyme name	No. Positions cuts of sites	Recognition sequence	More info
<i>Hae</i> II	2 248 265	rgcgcy	<a href="#">More info</a>

The following endonucleases were selected but don't cut this sequence:  
*Cl*I, *Pst*I

## 03RB1 [EU genotype]

### 484 base pairs

ctcgactaccaatacatatataatttgcgatatgtgagctaaacgggaccgctgctgctccgaccattttat base pairs  
gagctgatggttatgtatatattaacggctatacactcgatttgccttggcggaccgacagctgtaaaaata 1 to 75

tgggcagtcgagacctttgtgctctaccagtggcgactcacattttcactgggttcttaccgacaagccat base pairs  
acccgtcagctctgaaacacgagatgggtcaccgctgagtgtaagaaagtaccacaaaggaatgctgttcggta 76 to 150

tttttgatgactcgggtctcggggctgtgtctgttacaggattctatgacaagcggtagcttctcagcagctg base pairs  
aaaaactacgtgagccagagccccgacacagacaatgtcctaagatactgttcgcatgcaagagtcgtcgcac 151 to 225

*Hae*III  
ttttgctttgtcattagcgccttgcattgtttgttatccgtgctgtaaaaaattgtatggcttgcggttat base pairs  
aaaacgcaaacacgtaatcgtcgcgaacgtacaaaacaataggcacgacatttttaacataccgaacgcaata 226 to 300

gcccgtaccggtttaccacttcacgtggacgaccgggggagaatccatcggtggaggtctccatagtggtg base pairs  
cgggcattggccaaatggtgaagtagcactgctggcccccttaggtagccactccagaggggatcaccac 301 to 375

gaaaaattaggtaaagctgacgtcggcggcgacctgtcacatcaaacatgttgcctcaggaggagttaaagct base pairs  
cttttaaccatttcgactgcagccgctggaacagtggtatgttacaacaggagctccctcaatttcga 376 to 450

caaccctgacgaggacttcggccgagcaatggg base pairs  
gttgggaactgctcctgaagccgctgttacc 451 to 484

**Table by Enzyme Name**

Enzyme name	No. Positions cuts of sites	Recognition sequence	More info
<i>Hae</i> III	1 250	rgcgc/y	<a href="#">More info</a>

The following endonucleases were selected but don't cut this sequence:  
*Cl*I, *P*st



## 02BR1 [EU genotype]

### 496 base pairs

caacggcaccagctcgacataccaatacatatataattgccgatatgcgagctgaatgggaccgaatggttatc base pairs  
gttgccgtggtcgagctgtatggttatgtatatattaaacggctatacgtcgcacttacctggcttaccatag 1 to 75

cagccatttcattgggcagttgagacctttgtctttaccgggtgccaactcatattcttctactgggttctc base pairs  
gtcggtaaaagtaaccctgcaactctggaacacgaaatggccaacggtgagtataagaaagtgacccaaagga 76 to 150

*Pst*I  
cacaacgagtcattttttgacgcgctcggctcgggtgccgatccgctgcaggtattgttgccggcgggtatg base pairs  
gtgttctcagtaaaaaactgcgcgagccagagccacggcatagggacgctcctaaacaaccggcccatata 151 to 225

gctcagcagcatctacggcgtttgtcttcgcagcgttcgtatgtttgttatccgtgctgcaaaaaattcat base pairs  
cgagtctcgtagatgccgcaaacacgaaagcgtcgaagcatcaaaaataggcagcagcttttttaaagta 226 to 300

*Cl*aI  
ggcctgccgttatgcccgcaccggtttccaacttcattgtcgcagcaccgggggagagttatcgatggaagtc base pairs  
ccggacggcaatacggcgtggccaaatggtgaagtaacagctgctgccccctcaagtagctaccttcag 301 to 375

cccagtagtgtagaaaaattgggcaaagccgaagtcgacggcaacctgtcaccgtcaaacatgtcgtccttga base pairs  
gggtcatcaccatcttttaaccggttcggctcagctgccgttgaacagtggcagttgtacagcaggaact 376 to 450

aggggttaaagctcaaccctaacgaggacttcggccgagcaatgg base pairs  
tcccaatttcgagttggaattgctcctgaagccggctcgttacc 451 to 496

**Table by Enzyme Name**

Enzyme name	No. Positions cuts of sites	Recognition sequence	
<i>Cl</i> aI	1 364	at/cgat	<a href="#">More info</a>
<i>Pst</i> I	1 202	ctgca/g	<a href="#">More info</a>

The following endonucleases were selected but don't cut this sequence:  
*Hae*II

## EU-MLV vaccine 432 base pairs

gatggcaacggcaacagctcgacataccaatacatatataactgacgatatgcgagctgaatgggaccgactgg base pairs  
ctaccgttgccgttgctgagctgtatggttatgtatataatggaactgctatacgtcgaactaccctggctgacc 1 to 75

ttgtccagccattttgggtgggcagtcgagacctttgtgtttaccgggtgccactcatatcctctcactgggt base pairs  
aacaggtcggtaaaaccaaccgtcagctctggaacacaaaatggccaacggtgagtataggagagtgacca 76 to 150

*HaeII*      *PstI*  
tttccacaacaagccattttttgacgcgctcggctctgagcgttatccactgcaaggaatttggcggggcg base pairs  
aaagagtgttgcgtgtaaaaaactgcgcgagccagagccgcgacataggtgacgtcctaaacaaccgcccgc 151 to 225

*PstI*      *HaeII*  
tatgtactctgcagcgtctacggcgtgtgtcttcgcagcgttcgtatgtttgtcatccgtgctgctaaaaat base pairs  
atacatgagacgtcgcagatgccgcgaacacgaaagcgtcgcaagcatacaaaacagtaggcacgacatttta 226 to 300

*Clal*  
tgcattgacctgccgtatgcccgtaccgggttaccaacttcattgtagacaaccgggggagagttcatcgatgg base pairs  
acgtaccggacggcgatagcggcatgggccaatggtgaagtaacatctgttggccccctcaagtagctacc 301 to 375

aagtctccaatagtggtgaaaaattgggcaaagccgaagtcgacggcaacctgctc base pairs  
ttcagaggttatcaccatcttttaaccggttcggttcagctgcggttgagcag 376 to 432

**Table by Enzyme Name**

Enzyme name	No. Positions cuts of sites	Recognition sequence	
<i>Clal</i>	1 369	at/cgat	<a href="#">More info</a>
<i>HaeII</i>	2 194 251	rgcgc/y	<a href="#">More info</a>
<i>PstI</i>	2 207 238	ctgca/g	<a href="#">More info</a>

Every enzyme analyzed cuts this sequence.

## Vita

Miss Nusara punprapa was born on August 23,1976 at Klongsan district, Bangkok. She graduated from Faculty of Veterinary science, Chulalongkorn University on 1999. After graduated, she was appointed for training in the field of Veterinary Pathology and cell culture technique for 11 months at Department of Veterinary Pathology, Faculty of Agriculture, Miyazaki university, Japan which was financial supported by AIEJ foundation. Then, she entered the degree of Master of Science in Veterinary Pathobiology, Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University since 2001.



ศูนย์วิทยทรัพยากร  
จุฬาลงกรณ์มหาวิทยาลัย