การโคลนนิ่งและการศึกษาลำดับเบสซีดีเอ็นเอของตัวไวรับไซอะโลแอดฮีซินและซีดี 163 ที่แยกได้จากแมคโครฟาจสุกรที่ติดเชื้อไวรัสพีอาร์อาร์เอส

นายโว ฟอง วู ฮั้น ท้วน

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย
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เป็นแฟ้มข้อมูลของนิสิตเจ้าของวิทยานิพนธ์ที่ส่งผ่านทางบัณฑิตวิทยาลัย
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CLONING AND SEQUENCING OF SIALOADHESIN AND CD163 cDNA FROM PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS INFECTED PORCINE ALVEOLAR MACROPHAGES

Mr. Vo Phong Vu Anh Tuan

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Veterinary Medicine
Department of Veterinary Medicine
Faculty of Veterinary Science
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Thesis Title	CLONING AND SEQUENCING OF SIALOADHESIN AND CD163 cDNA FROM PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS INFECTED PORCINE ALVEOLAR MACROPHAGES
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โว ฟอง วู ฮ้น ท้วน : การโคลนนิ่งและการศึกษาลำดับเบสซีดีเอ็นเอของตัวไวรับไซอะโล แอดฮีซินและซีดี 163 ที่แยกได้จากแมคโครฟาจสุกรที่ติดเชื้อไวรัสพีอาร์อาร์เอส (CLONING AND SEQUENCING OF SIALOADHESIN AND CD163 cDNA FROM PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS INFECTED PORCINE ALVEOLAR MACROPHAGES) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ.น.สพ.ดร. อธิภู นันทประเสริฐ, 144 หน้า.

ไซอะโลแอดฮีซิน และ ซีดี 163 เป็นตัวไวรับอย่างน้อย 2 ชนิด ซึ่งพบที่แมคโครฟาจสุกรที่ติดเชื้อไวรัสพีอาร์อาร์เอส โดยมีความสำคัญต่อการสร้างภูมิคุ้มโรคในสุกรหลังจากได้รับเชื้อไวรัส วัตถุประสงค์ของงานวิจัยนี้ คือ การโคลนนิ่งและการศึกษาลำดับเบสซีดีเอ็นเอของตัวไวรับไซอะโลแอดฮีซิน (ทั้งสายและเฉพาะส่วน N-terminal) และ ซีดี 163 (ทั้งสายและเฉพาะส่วน domain 5) จากแมคโครฟาจของสุกรอนุบาลในประเทศไทยที่ติดเชื้อไวรัสพีอาร์อาร์เอสทั้ง 3 สายพันธุ์ เปรียบเทียบการแสดงออกของทั้งสองยีนกับยีน GAPDH เป็นตัวอ้างอิงภายใน การวิจัยนี้ได้ทำการสกัด RNA ทั้งหมดจากแมคโครฟาจ จำนวน 6 ตัวอย่างที่ติดเชื้อไวรัสพีอาร์อาร์เอสสายพันธุ์จีน สายพันธุ์ยุโรป และสายพันธุ์อเมริกา ต่อมาซีดีเอ็นเอของตัวไวรับไซอะโลแอดฮีซิน (ทั้งสายและเฉพาะส่วน N-terminal) และซีดี 163 (ทั้งสายและเฉพาะส่วน domain5) จะถูกเพิ่มปริมาณโดยใช้เทคนิค PCR เปรียบเทียบการแสดงออกของยีนจากความเข้มของ band ดีเอ็นเอที่ได้ จากนั้น ซีดีเอ็นเอของตัวไวรับจะถูกโคลนเข้าไปในเวคเตอร์ pCR-XL-TOPO® (ทั้งสาย) และ pCR8®-GW-TOPO® (เฉพาะส่วน N-terminal และ ส่วน domain 5) ทำการวิเคราะห์ และ เปรียบเทียบลำดับนิวคลีโอไทด์ และกรดอะมิโนรวมทั้งส่วนประกอบที่ได้ ผลการวิจัย พบว่า สามารถเพิ่มจำนวนซีดีเอ็นเอ และโคลนนิ่งยีนตัวไวรับทั้งสองเข้าสู่เวคเตอร์ทั้งสองชนิดได้สำเร็จโดยได้ไซอะโลแอดฮีซินทั้งสาย จำนวน 10 โคลน ซีดี 163 ทั้งสาย จำนวน 20 โคลน ไซอะโลแอดฮีซินเฉพาะส่วน N-terminal จำนวน 24 โคลน และ ซีดี 163 เฉพาะส่วน domain 5 จำนวน 29 โคลน เมื่อเปรียบเทียบส่วนประกอบ ลำดับนิวคลีโอไทด์ และกรดอะมิโนของซีดีเอ็นเอของตัวไวรับทั้งสองแล้ว พบว่า มีความคล้ายคลึงกันมากกว่า 95% นอกจากนี้ การแสดงออกของซีดีเอ็นเอของตัวไวรับทั้งสองชนิดที่แยกได้จากแมคโครฟาจสุกรอนุบาลในประเทศไทยที่ติดเชื้อไวรัสพีอาร์อาร์เอสสายพันธุ์อเมริกามีค่าสูงกว่าสายพันธุ์จีน และสายพันธุ์ยุโรป

ภาควิชา อายุรศาสตร์ ลายมือชื่อนิสิต .....
สาขาวิชา: อายุรศาสตร์สัตวแพทย์ ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก.....
ปีการศึกษา: 2554 ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

5375570431: MAJOR VETERINARY MEDICINE
KEYWORDS: CD163 / cDNA / PAM / PRRSV / RECEPTOR / SIALOADHESIN

VO PHONG VU ANH TUAN: CLONING AND SEQUENCING OF SIALOADHESIN AND CD163 cDNA FROM PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS INFECTED PORCINE ALVEOLAR MACROPHAGES. ADVISOR: ASSOC. PROF. ATHIPOO NUNTAPRASERT, Ph.D, 144 pp.

Sialoadhesin (Sn) and CD163 receptors were reported as two essential receptors on porcine alveolar macrophages (PAM) for PRRSV infection and importance for the study of the immunity of pigs after infection. The objectives of this study were cloning and sequencing the porcine Sn (full and N-terminal domain) and CD163 (full and domain 5) cDNA from 3 strains of PRRSV infected PAM of Thai nursery pigs and comparison of gene expression level with GAPDH gene as internal reference. In this thesis, total RNA was extracted from 6 positive EU, US and HP-PRRSV infected PAM. The porcine Sn (full and N-terminal domain) and CD163 (full and domain 5) cDNA were further amplified using PCR technique. Band densitometry was used to quantify expression level. Then, the cDNA were cloned into pCR®-XL-TOPO® (full cDNA) and pCR®8-GW-TOPO® (N-terminal domain and domain 5 cDNA) vectors. The nucleotide and deduced amino acid sequences including compositions were then analyzed and compared. The results showed that 10 clones of Sn (full) and 20 clones of CD163 (full) were constructed in pCR®-XL-TOPO® vectors, while 24 clones of Sn (N-terminal domain) and 29 clones of CD163 (domain 5) were transferred into pCR8®-GW-TOPO® vectors. The sequences and compositions of nucleotide and deduced amino acids of these genes from 3 strains of PRRSV were successfully analyzed and showed more than 95% similarity. Furthermore, the US strain of PRRSV infected PAM collected from Thai nursery pigs showed higher expression level of porcine Sn and CD163 cDNA than that of the HP and EU strains.

Department : Veterinary Medicine Student's Signature .....
Field of Study : Veterinary Medicine Advisor's Signature .....
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LIST OF ABBREVIATIONS

ASFV	=	African swine fever virus
aa	=	amino acid
bp	=	base pair
BHK-21	=	Baby hamster kidney
cDNA	=	Complementary DNA
CSF	=	Classical Swine Fever
DCs	=	dendritic cells
DNA	=	deoxyribonucleic acid
dNTP	=	dATP, dGTP, dTTP, dCTP
EAV	=	Equine Arteritis virus
ELISA	=	enzyme-linked immunoassay
ER	=	endoplasmic reticulum
EU	=	European genotype
FMD	=	Foot and Mouth Disease
GAPDH	=	Glyceraldehyde-3-phosphate dehydrogenase
GHR	=	Growth hormone receptors
GHRF	=	Growth hormone releasing factor
GP	=	Glycoprotein
Hb	=	hemoglobin
HP	=	High Pathogenic
Hp	=	haptoglobin
IFN	=	Interferon
IGFBP-5	=	Insulin-like growth factor binding protein-5
IGF-I	=	Insulin-like growth factor-I
IL	=	Interleukin
LB	=	Luria Bertani

LPS	=	Lipopolysaccharides
LV	=	Lelystad Virus
kDa	=	kilodalton
M	=	matrix protein
mRNA	=	Messenger ribonucleic acid
N	=	nucleocapsid protein
NCBI	=	National Center for Biotechnology Information
NSPs	=	non-structural proteins
ORFs	=	open reading frames
PAM	=	porcine alveolar macrophages
PBS	=	Phosphate buffered saline
PCR	=	polymerase chain reaction
PCV2	=	Porcine Circovirus type 2
PED	=	Porcine Epidemic Diarrhea
PK15	=	Porcine kidney 15
PRRS	=	porcine reproductive and respiratory syndrome
PRRSV	=	porcine reproductive and respiratory syndrome virus
RNA	=	Ribonucleic acid
rpm	=	Rounds per minute
RT-PCR	=	Reverse Transcriptase Polymerase chain reaction
SiRNA	=	Small inhibitory RNA
Sn	=	Sialoadhesin
SRCR	=	scavenger receptor cysteine-rich
TLR4	=	Toll-like receptor 4
TNF	=	Tumor necrosis factors
US	=	North American genotype
UV	=	Ultraviolet

CHAPTER I
INTRODUCTION

Many swine viruses can cause the diseases in pigs by replicating inside the target cells. Virus infection usually binds to its specific receptors on the surface of a target cell. The expressed receptor lets the virus to penetrate the cell, spread in the host, and develop the pathogenesis (Norkin, 1995). An important analysis of virus receptors is gained the knowledge for development of clinically effective antiviral agents. The recognition of virus receptor inhibitors of the viral receptor interaction has also useful for the study of viral infection. In addition, the fundamental, biological and clinical importance basis data of viral receptors may have the potential to design the drugs that limit the interaction between virus and its receptor at the initiation of viral binding and may suggest new therapy of viral diseases and the method to blocks viral infection into the target cell of host by using the other related viruses that use the similar receptor. Moreover, the inhibitors of the viral receptor interaction, siRNA functional genomics applications (Behlke, 2006) and the anti-receptor monoclonal antibodies may be clinically useful for inhibition of the replication of major viruses (Norkin, 1995). In swine diseases, there are many reports about viruses and their candidate receptors such as Heparan sulphate (Foot and Mouth Disease (FMD) virus) (Fry et al., 1999); Aminopeptidase N (Porcine Epidemic Diarrhea (PED) virus) (Li et al., 2007); Heparan sulphate and Chondroitin sulphate B (Porcine Circovirus type 2 (PCV2) virus) (Misinzo et al., 2006); Heparan sulphate (Classical Swine Fever (CSF) virus) (Hulst et al., 2000); Heparan sulphate, Sialoadhesin (Sn) and CD163 (porcine reproductive and respiratory syndrome (PRRS) virus) (Duan et al., 1997a; Duan et al., 1997b; Calvert et al., 2007). Among those diseases, PRRS is the most important disease on pig health and welfare worldwide more than 20 years after its emergence (Huang and Meng, 2010). Two receptors on porcine alveolar macrophages (PAM), which are porcine Sn and CD163, have been recognized for PRRS virus (PRRSV) entry and uncoating

(Vanderheijden et al., 2003; Calvert et al., 2007; Van Gorp et al., 2008). The PRRS virus is required these receptors to release viral genome into the cytoplasm of the host cell and spread the infection. Porcine Sn is considered as the attachment and internalization receptor (Vanderheijden et al., 2003). While at an early endosomes stage, the virus can liberate its genome by localization with CD163 (Van Gorp et al., 2009) which is a essential factor for PRRSV genome release (Van Gorp et al., 2008). Furthermore, the co-expression of Sn and CD163 receptors increases virus production and the efficiency of PRRSV infection (Van Gorp et al., 2008). Recently, porcine Sn and CD163 genes were reported to be related to the immunity of pigs after PRRSV infection and may be used to improve PRRS immunity of pigs (Wang et al., 2011). Therefore, the construction of recombinant porcine Sn or CD163 receptors produced by using bacterial system or mammalian system are useful for study of the process of PRRSV into the target cells (Calvert et al., 2007; Pérez et al., 2008; Van Gorp et al., 2008). Furthermore, the understanding of interaction between PRRSV and their receptors may be used to produce peptide mimetic, specific antibodies to block PRRSV entry and inhibit infection of pigs with the PRRSV (Das et al., 2010).

In this study, the porcine Sn (full and N-terminal domain) and CD163 (full and domain 5) receptor cDNA molecules isolated from PAM infected with 3 strains of PRRSV in Thai nursery pigs were cloned, expressed into a bacterial vectors, studied the nucleotide and deduced amino acid sequences. Briefly, the porcine Sn and CD163 receptor cDNA were amplified using polymerase chain reaction (PCR) technique with specific primers. The pCR®-XL-TOPO® vector containing kanamycin and pCR®-8-GW-TOPO® vector with spectinomycin resistant genes were used for construction the plasmids with porcine Sn and CD163 cDNA (full, N-terminal domain and domain 5); respectively. Those plasmids were then transformed into *E.coli*. Positive clone of transformants was selected and confirmed correction by using PCR technique. The sequences of study genes were studied and the deduced amino acids and homology were analyzed and compared with the available databases of the reference Sn (refSn) and the reference CD163 (refCD163) cDNA from the

PAM of normal pigs from GenBank. Furthermore, the expression level of porcine Sn and CD163 cDNA from the infected PAM with 3 strains of PRRSV were measured using densito-spectrophotometer and the relative density between these studied genes and housekeeping gene (GAPDH) were analyzed and compared using FusionCapt Advance SL4 machine and Bio 1D software.

The cloning and sequencing of porcine Sn and CD163 cDNA from PRRSV infected PAM in Thai nursery pigs is the first study in Thailand. The knowledge from this study may be useful for understanding these two PRRSV receptors. Their nucleotide and deduced amino acid sequences and compositions may be applied for construction of these recombinant receptors and may helpful to inhibit and control PRRSV infection in pigs in the future.

CHAPTER II
LITERATURE REVIEW

2.1 Genome of Porcine reproductive and respiratory syndrome virus (PRRSV)

PRRSV is enveloped, single stranded and positive sense RNA virus. PRRSV is belonged to the family *Arteriviridae* and the order *Nidovirales* based on the similarity of their genome structure and replication strategy (Snijder and Meulenberg, 1998). It consists of nine open reading frames (ORFs) as shown in Figure 2.1. All of the ORFs were overlap with each other except between ORF1b and ORF2a. ORF1a and ORF1b (Snijder and Meulenberg, 1998) together span roughly 75% of the genome from the 5'-end. These ORFs make glycoproteins that are processed by different viral proteases to produce a total thirteen to fourteen non-structural proteins (NSPs), named NSP1α, NSP1β, and NSP 2-6, NSP7α, NSP7β, and NSP8-12, respectively (Van Aken et al., 2006; Kroese et al., 2008). The ORFs 2a, 2b, 3-7 contain about 25% of the genome at the 3'-end and they encode the structural proteins, namely, the glycoprotein (GP) 2a (GP2a), 2b (or E), GP3, GP4, GP5, the matrix protein (M), and the nucleocapsid protein (N), respectively (Figure 2.2) (Wu et al., 2005). GP2a, GP3, GP4, and GP5 are N-glycosylated and are present on the viral envelope (Dea et al., 2000) as are the non-glycosylated M protein and 2b proteins. GP5, M, and N proteins are known as major structural proteins whereas GP2a, 2b (E), GP3, and GP4 proteins are regarded as minor structural proteins. All of the minor and major surface proteins are requisite for production of PRRSV infection.

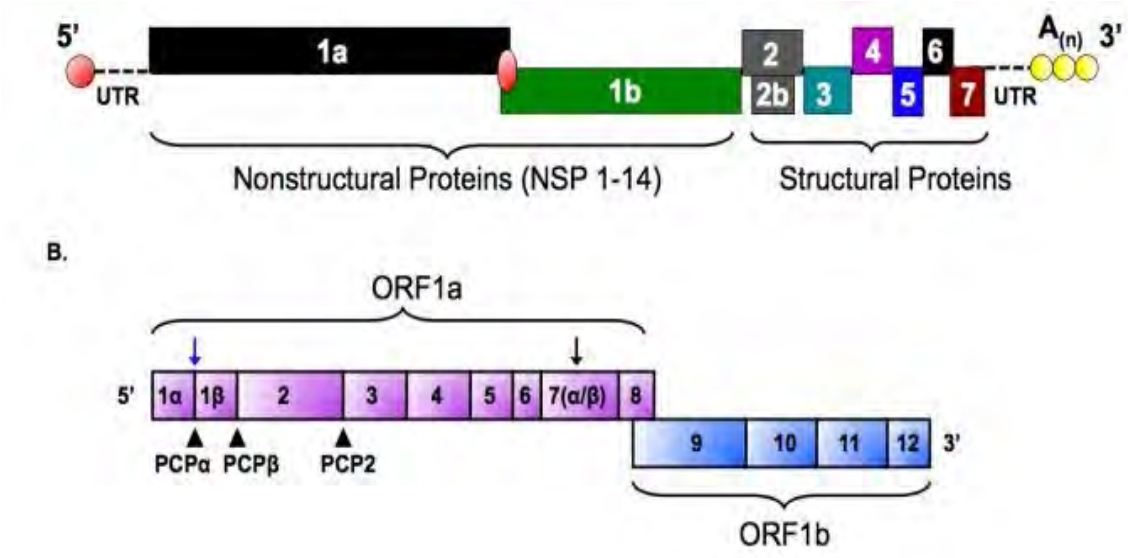

Figure 2.1 Diagram representation of PRRSV genome organization (Das et al., 2010)

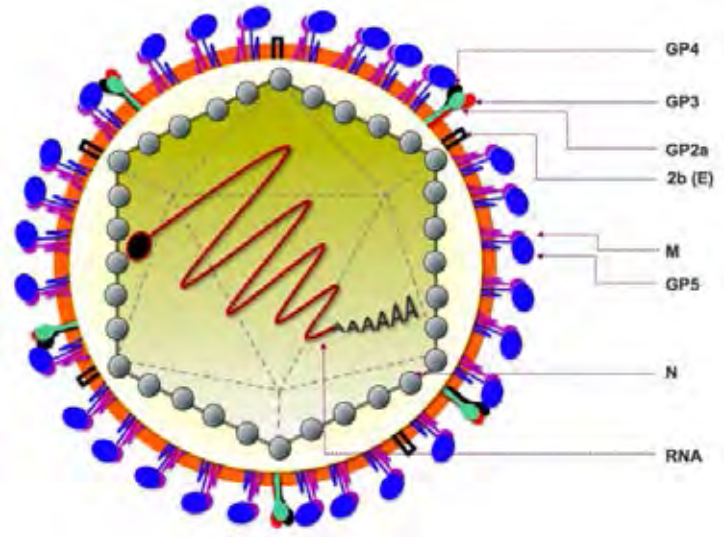

Figure 2.2 Diagram representation of PRRSV particle structure (Das et al., 2010)

2.2. PRRSV strains

Previously, PRRSV can be categorized in genotype based on the same genotypes, common and type specific antigenic determinant present on the structural proteins (Dea et

al., 2000; Forsberg, 2005; Hanada et al., 2005). It has two prototype strains. Genotype I (European genotype) and genotype II (North American genotype) were distinguished based on 40 % dissimilarities of genome sequence homology. These two genotypes were also separate serologically (Kim and Yoon, 2008). The North American strain is VR-2332 (Nelsen et al., 1998) and the European strain is the Lelystad Virus (LV) (Meulenberg et al., 1997). Recently, the new PRRSV strain, as China strain or High Pathogenic (HP) strain (related to the North American PRRSV genotype) is known for rapid and severe spread among Asian countries (Li et al., 2007). The European and North American PRRSV strains cause the same of clinical symptoms, but they are different viral genotypes whose genomes separate by about 37 % (Allende et al., 1999), thus creating a covering of obscurity about the this virus origin. HP-PRRSV strain is greatly pathogenic infectious disease that firstly emerged in pigs in the central region of China (Li et al., 2007; Tian et al., 2009). This HP-PRRS was showed by a prolonged high fever of above 41^{o}C, red discoloration of the ears and body, anorexia, and high mortality in later periods.

2.3 PRRSV cell tropism

The target cells of PRRSV were studied (Duan et al., 1997**).** However, the porcine alveolar macrophages are primary target cells for the PRRSV infection (Duan et al., 1997; Teifke et al., 2001). PRRSV mostly infects alveolar macrophages of the lung and does not replicate in non-activated monocytes (Thanawongnuwech et al., 2001). Besides primary porcine alveolar macrophages, PRRSV only in vitro infect into the African green monkey kidney cells (MA-104), and the derived cells such as Marc-145 cells (Kim et al., 1993), but those cell lines do not express Sn (Kim et al., 1993; Calvert et al., 2007). From the literature, it is suggesting that porcine alveolar macrophages (PAM) are suitable for studying PRRSV receptors.

2.4. Essential PRRSV receptors

2.4.1. Porcine Sialoadhesin

The Sialoadhesin (Sn), known as CD169 or Siglec-1, is a type I membrane protein (Williams and Barclay, 1988). Sn was initially known as a sialic acid-dependent sheep erythrocyte receptor on resident bone marrow cells of rats and was also characterized in human, mice and swine (Crocker and Gordon, 1985; Vanderheijden et al., 2003). Sn is member of the family of sialic acid binding immunoglobulin-like lectins (siglecs) (Crocker and Gordon, 1985). The siglecs are type I membrane proteins displaying an amino-terminal V-set immunoglobulin domain that binds sialic acid and variable numbers of downstream C2-set immunoglobulin domains (Crocker et al., 2007). The molecular characterization of Sn indicated that it had 17 immunoglobulin domains of extracellular region, a feature that was well conserved in mammals and its cytoplasmic tail was poorly conserved (Crocker et al., 1994). The extracellular region of Sn could be subdivided into a single amino-terminal V-set domain and 16 C2-set domains (Figure 2.3). The 16 C2 repeats ensure that the terminal V-set domain was kept clear of sialic acid residues on the macrophage while active towards sialic acid conjugated on target cells (Munday et al., 1999).

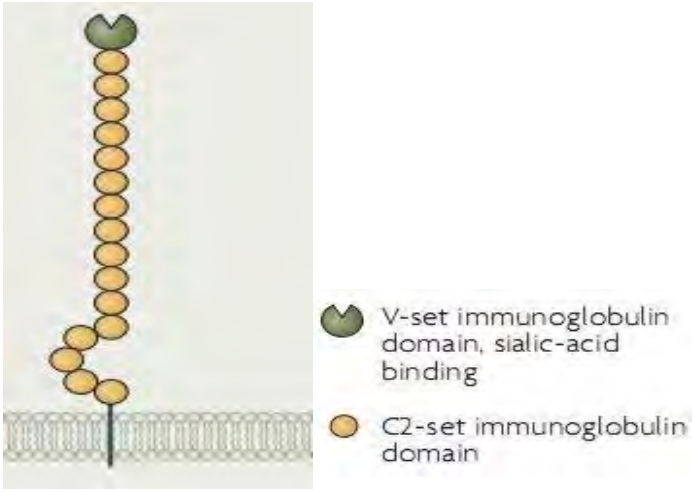

Figure 2.3 Diagram representation of Sn domain (Crocker et al., 1994)

Sn expression was found only on specific subsets of tissue macrophages. Immunocytochemical staining revealed that Sn was constitutively expressed on subpopulations of tissue-resident macrophages, especially those in secondary lymphoid organs (Crocker and Gordon, 1989). Using antibodies raised to the recombinant protein, the expression pattern of human Sn was found to be similar to that of the mouse receptor. Sn was obtained absent from monocytes and other peripheral blood leukocytes, but expressed strongly by tissue macrophages in the spleen, lymph node, bone marrow, liver, colon, and lungs. High expression was also found on inflammatory macrophages presented in affected tissues and was rapidly upregulated by inflammatory macrophages (Hartnell et al., 2001). In contrast, using immunocytochemistry to examine the resident macrophage populations of the nervous system, it showed that Sn was not expressed in the microglia (Perry et al., 1992).

The functions of Sn were suggested as regulators of adhesion, endocytosis and pro-inflammatory (Crocker et al., 2007). It has been reported that the Sn role was as an adhesion molecule for lymphocytes and that it was involved in the modulation of lymphocyte activity (Umansky et al., 1996). Unlike most Siglecs, Sn lacks tyrosine-based signaling motifs and its cytoplasmic tail is poorly conserved, which suggests a primary role as a binding partner in cell–cell interactions, rather than in cell signaling (Crocker et al., 1994). It is recently show that under normal conditions, Sn-deficient mice revealed only delicate changes in the haematopoietic and immune systems (Crocker et al, 2007). However, in a model of peptide-induced experimental autoimmune uveoretinitis, the lacking mice showed reduced retinal inflammation and T cells isolated from draining lymph nodes indicated lowered proliferative responses in vitro (Jiang et al., 2006). Furthermore, in two genetically determined models of peripheral and central nervous system demyelination, disease in Sn-deficient mice was improved and numbers of infiltrating CD8+ T cells and macrophages at the sites of inflammation were reduced (Ip et al., 2007). These new findings were consistent with a potentially important role of Sn in modulating T-cell function and activation during

immune responses (Crocker et al., 2007). An additional possibility was that Sn functioned as a phagocytic receptor to clear sialylated pathogens. The cellular and molecular bases for these effects suggested that Sn could mediate both sialic-acid-dependent and sialic-acid-independent interactions with cells of the immune system. Sialic-acid-dependent Sn interactions might be mediated by mucin-like molecules presenting high densities of sialylated O-linked glycans. Sialic-acid-independent Sn interactions could involve the mannose receptor and macrophage galactose-type N-acetylgalactosamine-specific lectin 1 (Kumamoto et al., 2004). The membrane lectins were expressed on dendritic cells (DCs) and have been shown to bind Sn extracted from lymphoid tissues. Although DCs themselves do not normally express Sn, it could be induced on human monocyte-derived DCs following exposure to rhinoviruses in vitro (Kirchberger et al., 2005). Interestingly, these DCs were poor stimulators of T cells in mixed lymphocyte reactions, a feature that was partly attributed to the expression of Sn. It is possible that when Sn is expressed by macrophages it is immunostimulatory, whereas on DCs it is immunosuppressive (Kirchberger et al., 2005).

The porcine Sn is imperative for its function as the PRRSV receptor (Crocker et al., 1994; Van Breedam et al., 2010). The porcine Sn gene is 5,193 bp in length and encodes a large protein of 210 kDa (Delputte et al., 2007) and contains 1,730 amino acids (NP_999511, GenBank). The porcine Sn was considered as the attachment and internalization receptor since expression of this protein in non-permissive mammalian cells lead to binding and internalization of PRRSV but not productive viral infection (Vanderheijden et al., 2003). Using monoclonal antibody (41D3) that is specific for porcine Sn, which PRRSV infection is blocked, inhibited this interaction. These findings indicated that Sn is sialic acid binding lectin and that communication between sialic acid on the PRRSV particle and Sn are essential for PRRSV infection of PAM. Similarly, Vanderheijden et al (2003) reported that Sn is concerned in the entry of PRRSV into PAM. The role and co-operation of heparan sulphate and Sn during PRRSV attachment and internalization was

analyzed (Delputte and Nauwynck, 2004). These results revealed that heparan sulphate was not necessary for Sn to function as a PRRSV internalization receptor. In the study, whether the N-terminal domain of Sn is sufficient and/or necessary for PRRSV attachment. An et al. (2010) constructed a chain of truncated segments of Sn and expressed these in the non-permissive PK15 cell line (An et al., 2010). The result shown that the first domain at the N-terminal Sn mediates PRRSV binding to porcine alveolar macrophage cells and contributed to improved understanding the interaction between PRRSV and its target cells. Most recent, the major M/GP5 glycoprotein complex of PRRSV was identified as a ligand for Sn through identifying the viral counterparts for Sn by constructing and validating a soluble form of Sn (Van Breedam et al., 2010). PRRSV has only been obtained to infect and its replication was high levels in MARC-145 cells (Kim et al., 1993), a derived of the African green monkey kidney cell line MA-104. Nevertheless, Sn is absent on MARC-145 cells (Kim et al., 1993; Duan et al., 1998; Wissink et al., 2005) indicating that the molecules other than Sn promote entry of PRRSV in MARC-145 cells. In addition, porcine CD163 obtained from PAM cells was discovered to confer susceptibility to infected PRRSV to non-permissive cells (Calvert et al., 2007).

2.4.2. Porcine CD163

The CD163 is a type I transmembrane protein and is member of scavenger receptor cysteine-rich (SRCR) superfamily (Calvert et al., 2007). The SRCR superfamily are characterized by a cysteine-rich domain (SRCR domain), homologous to the C-terminus of the type I macrophage scavenger receptor (Freeman et al., 1990). Most of the SRCR superfamily proteins are found on cells associated with the immune system (Resnick et al., 1994), but some of the members are also found on other cells, such as hepatocytes (Goldberger et al., 1987) and epithelial cells of the gastrointestinal tract (Li and Snyder, 1995). Functionally, the SRCR domains are thought to mediate protein–protein interactions

and ligand binding (Krieger and Herz, 1994; Pearson, 1996). The SRCR domain has a 100–110 amino acid residues (Resnick et al., 1994). Proteins with SRCR domains are divided into two groups (A and B) based on the localization and number of cysteine residues. Group A all have six cysteine residues per domain but lack cysteine residues at positions 1 and 4. Members of group B have either six or eight cysteine residues per domain, but the cysteine residues at positions 1 and 4 are always present (Resnick et al., 1994). It is identified that CD163 is a group B SRCR protein containing eight cysteine residues per domain, but with only six cysteine resides in domain 8 (Nielsen et al., 2006). Structurally, CD163 has an ectodomain consisting of 9 SRCR domains in tandem. The basic transcript encodes for a protein of 1076 amino acids. The extracellular part contains 1003 amino acids. The transmembrane single segment consists of 24 amino acids. Short cytoplasmic domains consist of 49 amino acids (Ritter et al., 1999; Nielsen et al., 2006). A short linker section then links SRCR domain 9 with a transmembrane domain and an intracellular cytoplasmic tail. Five different isoforms of CD163 have been described so far. They differ in the structure of their cytoplasmic domains and putative phosphorylating sites. Three of these isoforms display different splicing forms of the cytoplasmic domain, which vary from 49 to 84 or 89 amino acids, respectively. The first 42 amino acids after the membrane spanning segment are common for all three isoforms (Nielsen et al., 2006). Two possible alternatives splice sites are observed in an extracellular part of the molecule, one generating a stop codon resulting in a truncated form of protein only consisting of the first three SRCR domains, the other introducing additional 33 amino acids between SRCR 5 and 6 domains (Vilà et al., 2000).

Expression of CD163 is restricted to cells of the monocyte/macrophage lineage. In tissues, CD163 staining is predominantly observed on resident tissue macrophages such as red pulp macrophages in the spleen, Kupffer cells in the liver, and interstitial and alveolar macrophages in the lungs (Fabriek et al., 2005). In addition, the CD163 antigen is present on perifollicular and medullary macrophages in lymph nodes, perifollicular macrophages in

the tonsils, medullary and cortical macrophages in the thymus, and perivascular and meningeal macrophages in the central nervous system (not microglia) (Sánchez et al., 1999; Van den Heuvel et al., 1999). The expression of CD163 is regulated by a variety of factors. Expression of CD163 could correlate with the degree of macrophage activation, because newly infiltrating macrophages are CD163-negative but up-regulate their expression during the healing phase of acute inflammation, in chronic inflammation, and also in wound-healing tissue (Sulahian et al., 2000; Williams et al., 2002). CD163 expression is strongly up-regulated by anti-inflammatory inducers (such as glucocorticoids, IL-6, and IL-10) and down-regulated by proinflammatory agents (such as LPS, TNF-α, and IFN) (Högger et al., 1998; Buechler et al., 2000). However, the mechanisms of CD163 expression are regulated in a complex way: for example, LPS binding to TLR4 induces expression of IL-6 and IL-10, which in turn induce CD163 expression (Weaver et al., 2007). LPS is then able to indirectly induce expression of CD163. Likewise, reduction in CD163 expression mediated by LPS is not a consequence of synthesis inhibition or increased turnover, but rather of induced ectodomain shedding (Weaver et al., 2007).

The role of CD163 has been suggested during erythropoiesis because of its reported ability to bind erythroblasts and promote erythroid expansion in vitro (Fabriek et al., 2007). CD163 was shown to directly interact with erythroblastic cells and a thirteen amino acid motif in the second SRCR domain of CD163 was identified to mediate this binding. Interaction of this CD163 motif with erythroblasts promotes the growth and/or survival of these cells (Fabriek et al., 2007). CD163 was also identified as the endocytic receptor binding hemoglobin (Hb) in complex with the plasma protein haptoglobin (Hp). This specific receptor ligand interaction leading to removal from plasma of the Hp–Hb complex, but not free Hp or Hb, now explains the depletion of circulating Hp in individuals with increased intravascular hemolysis. Besides having a detoxificating effect by removing Hb from plasma, the CD163-mediated endocytosis of the Hp–Hb complex may represent a major pathway for uptake of iron in the tissue macrophages (Graversen et al., 2001). Moreover,

CD163 proposes a role for this molecule as innate immune sensor for bacteria. CD163 has been shown to bind both gram-positive and gram-negative bacteria (Fabriek et al., 2009). Expression of CD163 in monocytic cells promoted bacteria-induced production of pro-inflammatory cytokines, like TNF-α. CD163 is suggested to act as an innate immune sensor for bacteria and inducer of local immunity, rather than as a phagocytic receptor (Fabriek et al., 2009). For other pathogens however, like the porcine viruses ASFV and PRRSV, CD163 serves as a portal that allows infection of their target cells belonging to the monocyte/macrophage lineage (Sánchez-Torres et al., 2003; Van Gorp et al., 2008; Patton et al., 2009).

Porcine CD163 also functions as a cellular receptor for PRRSV (Calvert et al., 2007). The porcine CD163 gene has 3,400 bp in length and encodes a large protein of 150 kDa (Delputte et al., 2007) with 1,133 amino acids (NP_999141, GenBank). Porcine CD163 recognized from PAM cells was obtained to confer susceptibility for PRRSV infection to non-permissive cells (Calvert et al., 2007; Das et al., 2010). Furthermore, porcine CD163 is expressed in MARC-145 cells and cells of monocyte and macrophage lineage that facilitating entry of PRRSV in these cells. Calvert et al. (2007) found that the CD163 expression from PAM, human cells (histiocytic lymphoma), African green monkey kidney cells (MARC-145 and Vero), primary mouse peritoneal macrophages, and canine (histocytosis) cells involved in encoding functional PRRSV receptors. Moreover, porcine kidney (PK032495), feline kidney (NLFK), or baby hamster kidney (BHK-21) cell lines were recorded as the parental cell lines that were susceptible to PRRSV infection (Calvert et al., 2007). Recombinants of CHO cells stably expressing porcine CD163 have been used for the description of three new monoclonal antibodies against porcine CD163. They used enzyme-linked immunoassay (ELISA) to evaluate levels of soluble CD163 in porcine sera and biological fluids (Pérez et al., 2008). Flow cytometry analysis was used to study the expression of porcine CD163 on PAM (Patton et al., 2009). The findings indicated that the levels of expressed porcine CD163 well correlated with the generally level of PRRSV

copying. These data also showed that the CD163 of expression in dissimilar microenvironments on macrophages (in vivo) can ascertain the replication effectiveness and following pathogenesis of PRRSV. The similar study was carried out (Lee et al., 2010) and the outcomes showed that PAM cell lines expressed significant levels of porcine CD163 and were entirely permissive for both EU and US PRRSV strains. The porcine CD163 can enhance virus replication (Lee et al., 2010). The porcine CD163 protein domains concerned in PRRSV infection were identified based on created deletion and chimerical mutants. The infection experiments showed that scavenger receptor cysteine-rich (SRCR) domain 5 (SRCR 5) is essential for PRRSV infection (Van Gorp et al., 2010) and porcine CD163 enhanced virus replication with significant increases in viral protein synthesis and progeny release (Jin Lee and Changhee Lee, 2010). The GP2a and GP4 protein were critical for mediating interglycoprotein interactions (Figure 2.4) (Das et al., 2010) and serves as the viral binding protein that is reliable for intermediate interactions with porcine CD163 for virus infection into susceptible host cell (Das et al., 2010). To identify the porcine CD163 protein domains involved in PRRSV infection, the deletion mutants and chimeric mutants were created. These results showed that scavenger receptor cysteine-rich (SRCR) domain 5 (SRCR 5) is essential for PRRSV infection, while the four N-terminal SRCR domains and the cytoplasmic tail are not required (Van Gorp et al., 2010). The remaining porcine CD163 protein domains need to be present but can be replaced by corresponding SRCR domains from CD163-L1, resulting in reduced (SRCR 6 and interdomain regions) or unchanged (SRCR 7 to SRCR 9) infection efficiency (Van Gorp et al., 2010).

Figure 2.4 Diagram representation of PRRSV glycoproteins and porcine CD163 receptor (Das et al., 2010)

Taken together, the essential PRRSV receptors from review literature are porcine Sn (full and N-terminal domain) and porcine CD163 (full and domain 5). These two receptors were suitable for further studied.

CHAPTER III
MATERIALS AND METHODS

Framework of the study

The framework of this study is shown in Figure 3.1. In this study, a history of a PRRS clinical outbreak farm was used for selecting PRRSV-infected farm. The sera and PAM were collected from nursery pigs with PRRS clinical signs. Then, the molecular technique (RT-PCR) was confirmed the strains of virus from positive PRRSV-infected nursery pigs. The expression and cloning of porcine Sn (full and N-terminal domain) and CD163 (full and domain 5) cDNA were performed and were compared after their total RNA isolated from PRRSV infected PAM samples. The sequences and compositions of recombinant plasmids and deduced amino acids were studied and compared homology with available database for insight information from GenBank.

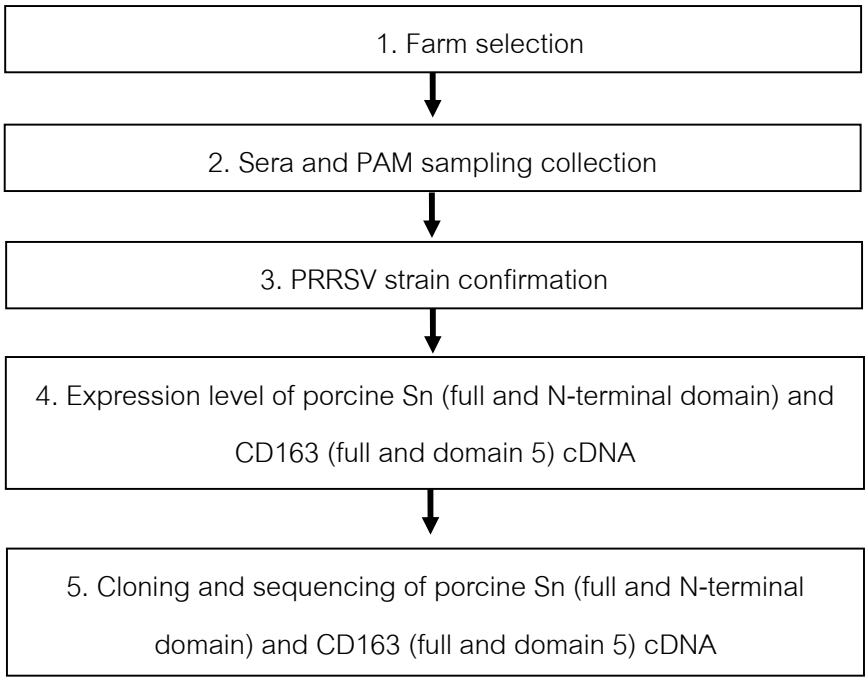

Figure 3.1 Framework of the study

Description of the study

Study farm

Six PRRS outbreak farms (Table 3.1) from the central part of Thailand (Ratchaburi province) were investigated based on clinical signs of PRRSV infection such as abortion and late-term reproductive failure in sows, respiratory disease in nursery and fattening pigs, etc (Li et al., 2007). The candidate farm scale with the sows range from 1,000 to 5,000 was selected.

Table 3.1 Number of Farms, nursery pigs and PAM samples

The positive PRRSV Farms (strain)	Nursery Pigs (3 pigs/farm)	PAM samples
2 (EU strain)	6	2
2 (US strain)	6	2
2 (HP strain)	6	2
Total: 6	**18**	**6**

Sample collection

The eighteen nursery pigs (three nursery pigs/farm) were obtained from those six pig farms as shown in Table 3.1. Five milliliters of sera were collected from each nursery pig with a PRRS clinical sign and stored at -20^{o}C until use. After sera collection, the nursery pigs were euthanized and lungs were collected. The PAM samples were collected by washing broncho-alveolar lung with sterile phosphate buffered saline (PBS) three times with a total volume of approximately 50 ml of PBS. The harvested wash fluid was then centrifuged for 10 minutes at 1,000 rpm. The final cell pellet was re-suspended in 5 ml of

PBS, and the number of PAM was counted to adjust the cell concentration (1 x 10^6 cells/ml). The PAM was kept fresh and all samples must be processed as soon as possible to maximize the chance of obtaining good quality of RNA. The six PAM samples from the positive PRRSV infected nursery pigs were named as shown in Table 3.2 and used for followed study.

Table 3.2 Sample name of PRRSV infected PAM

Iterms	PRRSV strain	Sample name
1	HP	Thai-HP-1; Thai-HP-2
2	EU	Thai-EU-1; Thai-EU-2
3	US	Thai-US-1; Thai-US-2

Phase I. Confirmation of positive PRRSV pig farms

The studied Thai nursery pigs were confirmed positive PRRSV infection from sera samples using RT-PCR and ELISA techniques. The PRRSV strains were confirmed by using RT-PCR (Eglia et al., 2001; Vo and Nuntaprasert, 2011) with positive control (PRRSV of ATCC VR2332 for US strain, ATCC LV for EU strain, CH1-a Vaccine for HP strain; respectively) and negative control (DEPC water). The S/P ration of PRRSV immune response was measured by using ELISA kit (IDEEXX, USA).

1. Total RNA isolation

Total RNA extractions were performed in a clean and separate area to minimize the chance of cross-contamination. The procedures of total RNA isolation were performed following the protocol of commercial kit's introduction (PureLink™ Viral RNA/DNA Kit,

Invitrogen, USA). According to the protocol, sera samples were added proteinase K and lysis buffer. Next, the mixture was incubated at 56°C for 15 minutes. After that, total RNA were bound onto the Spin Column and then washed with W5 Buffer (contains ethanol). Finally, total RNA was eluted by RNase free water.

2. Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) two-step protocols

2.1 cDNA synthesis

The RT-PCR kit being used was SuperScript™ RT-PCR System (Invitrogen, San Diego, CA, USA) containing SuperScript II Reverse Transcriptase. Briefly, this step used specific chemicals. The mixture of total RNA, Oligo (dT) and dNTP were incubated at 65°C for 5 minutes and quick chill on ice. The cDNA synthesis mix (RT buffer, $MgCl_2$, DTT, RNaseOUT and SuperScript) were added to the RNA mixture. Then, the contents of the tube was mixed by pipetting gently up and down and incubated at 50°C for 50 minutes. The final reaction was inactivated by heating at 85°C for 5 minutes. The first-strand cDNA was used as a template for amplification in PCR. The 5 µl of cDNA template were mixed with a reaction mixture containing 1 µl of each specific primer (10 µM), 2 µl of 10X PCR Buffer, 2 µl of 10 mM dNTP Mix, 0.5 µl of Pfu-Taq DNA polymerase (5 U/µl) and 8.5 µl of nuclease-free water (total volume reaction of 20 µl).

2.2 Primers and conditions used for PCR

The reverse-transcribed into cDNA of ORF1 and ORF7 was performed in a one-tube of 20 µl in volume (Eglia et al., 2001; Vo and Nuntaprasert, 2011). The two pairs of primers to amplification of ORF1 and ORF7 PRRSV were shown in Table 3.3 and the sizes of specifically amplified products are 756 bp (US strain), 666 bp (HP strain) and 478 bp (EU strain), respectively.

PCR condition was shown in Table 3.4. The PCR products on agarose gel electrophoresis of 1.5 % were analyzed and readily visible by UV transillumination with an ethidium bromide-stained gel.

Table 3.3 Nucleotide sequences of PCR primers used for RT-PCR

Gene	Sense	Sequence (5' to 3')	GenBank	Predicted product sizes (bp)
1. Sn (full)	+	ATGGACTTCCTGCTCCTGCTC	EU003993	5,193
	-	TCAGACTGTGCTTTTCACAGA		
2. CD163 (full)	+	ATGGTGCTACTTGAAGACTCT	NM_213976	3,400
	-	TAGTCCAGGTCTTCATCAAGG		
3. Sn (N-terminal)	+	ATGCTCCTGGCTTCATCTGC	EU003993	1,024
	-	TCCACCTCCATGCCCTCATG		
4. CD163 (domain 5)	+	GGACATTCCCTGCTCTGGTC	NM_213976	395
	-	CCATGTCCCAGTGAGAGTTG		
5. NSP 2 (full)	+	AAAGACCAGATGGAGGAGGA	GU454850	756
	-	GAGCTGAGTATTTTGGGCGTG		
6. NSP 2 (deleted)	+	AAAGACCAGATGGAGGAGGA	GU454850	666
	-	GAGCTGAGTATTTTGGGCGTG		
7. ORF 7	+	ATGCCAAATAACAACGGCAAG	GQ330474	478
	-	TCATGCTGAGGGTGATGCTGT		
8. GAPDH	+	TCAATGGAAATCCCATCACC	AF017079	720
	-	TGACAAAGTGGTCGTTGAGG		

Phase II: Expression of porcine Sn (full and N-terminal domain) and CD163 (full and domain 5) cDNA

This study used the PAM samples (as shown in Table 3.1) from positive PRRSV infected Thai nursery pigs for expression of porcine Sn (full and N-terminal domain) and CD163 (full and domain 5) cDNA. Gene expression level of porcine Sn (full and N-terminal domain) and CD163 (full and domain 5) cDNA were synthesized using a reverse transcription-polymerase chain reaction (RT-PCR) technique (Lee et al., 2002). The housekeeping gene named glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a reference control (Foss et al., 1998 ; Suzuki et al., 2000).

1. Total RNA isolation from PAM samples

Total RNA was extracted from total 6 PAM samples of positive 3 strains of PRRSV infected Thai nursery pigs using the protocol of commercial Kit's introduction (Total RNA Minikit, Geneaid, Taiwan). According to the protocol, PAM samples ($1x10^6$ cells/ml) were efficiently homogenized in a micro-centrifuge tube. The cells were lysed by using RB buffer and β-mercaptoethanol. The total RNA were bound on RB column and then washed with W1 buffer and then were eluted in sterile with RNase free water. The concentration of total RNA was measured by using Nanodrop ND-1000 (Thermo Scientific, Wilming-Ton, DE, USA).

2. Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Five µg of total RNA from 3 strains of PRRSV infected PAM were reversibly transcribed to cDNA using SuperScript™ RT-PCR System (Invitrogen, San Diego, CA, USA). This RT-PCR kit contains SuperScript II Reverse Transcriptase. Briefly, this step used specific chemicals. The mixture of RNA, Oligo (dT) and dNTP were incubated at 65°C for 5

minutes and quick chilled on ice. The cDNA synthesis mixture (RT buffer, $MgCl_2$, DTT, RNaseOUT and SuperScript) were added to RNA mixture. Then, the contents of the tube were mixed by pipetting gently up and down and then incubated at 50°C for 50 minutes. Finally, the reaction was inactivated by heating at 85°C for 5 minutes. The cDNA were used as a template for amplification in PCR step. The concentration of cDNA was measured by using Nanodrop ND-1000 (Thermo Scientific, Wilming-Ton, DE, USA).

3. Primers and conditions used for PCR

The 5 µl of cDNA templates were mixed with a reaction mixture containing 1 µl of each specific primers (10 µM), 2 µl of 10X PCR Buffer, 2 µl of 10 mM dNTP Mix, 0.5 µl of Pfu-Taq DNA polymerase (5 U/µl) and 8.5 µl nuclease-free water (total volume reaction of 20 µl). The specific primers were shown in Table 3.3 and the PCR condition (Vanderheijden et al., 2003**;** Pérez et al., 2008; Vo and Nuntaprasert, 2012**)** was shown in Table 3.4. PCR for GAPDH was also done followed the previous studies (Foss et al., 1998; Suzuki et al., 2000).

4. The gene expression level

The PCR products were stained with ethidium bromide in 1.5 % agarose gel electrophoresis and analyzed by using UV transillumination. A FusionCapt Advance SL4 (Vilber Lourmat, Germany) was used to capture the image and using Bio 1D Advance software to analyze density of the expressing bands of porcine Sn and CD163 cDNA. The expression level of porcine Sn and CD163 cDNA were compared with GAPDH housekeeping gene.

Table 3.4 PCR condition program

cDNA	1 cycle	35 cycles			1 cycle	Hold
	Denaturation	Denaturation	Annealing	Extension	Extension	
1. NSP2&ORF 7	94°C (2 minutes)	94°C (30 seconds)	60°C (20 seconds)	72°C (1 minute)	72°C (5 minutes)	4°C
2. Sn (full)	94°C (5 minutes)	94°C (30 seconds)	61°C (30 seconds)	72°C (5 minutes and 20 seconds)	72°C (10 minutes)	4°C
3. Sn (N-terminal domain) & GAPDH	94°C (2 minutes)	94°C (20 seconds)	62°C (20 seconds)	72°C (1 minute)	72°C (5 minutes)	4°C
4. CD163 (full)	94°C (5 minutes)	94°C (30 seconds)	63°C (30 seconds)	72°C (3 minutes and 40 seconds)	72°C (10 minutes)	4°C
5. CD163 (domain 5) & GAPDH	94°C (2 minutes)	94°C (20 seconds)	62°C (20 seconds)	72°C (1 minute)	72°C (5 minutes)	4°C
6. GAPDH	94°C (2 minutes)	94°C (20 seconds)	62°C (20 seconds)	72°C (1 minute)	72°C (5 minutes)	4°C

Phase III. Cloning and sequencing of porcine Sn (full and N-terminal domain) and CD163 (full and domain 5) cDNA

1. Cloning of porcine Sn (full) and CD163 (full) cDNA

The corrected size of PCR products were cut from the agarose gels. These gel bands were then purified by using Gel/PCR DNA Fragments Extraction Kit (Geneaid Company, Taiwan). The gel purified PCR products of porcine Sn (full) and CD163 (full) were measured by using Nanodrop ND-1000 (Thermo Scientific, Wilming-Ton, DE, USA). At the first time, the author cloned these gene receptors directly into the pCR®-8-GW-TOPO® vector using pCR®-8-GW-TOPO® TA Cloning Kit (Invitrogen, USA) according to the manufacturer's instructions. However, no positive colony was detected. Then, the pCR®-XL-TOPO® vector with TOPO®-XL-PCR Cloning Kit (Invitrogen, USA) were chosen for long length (3.0 kb – 10.0 kb) PCR products insertion. Briefly, the TOPO® cloning reaction for eventual transformation into either chemically competent cells were performed in a tube of 6 µl in volume. The mixtures of fresh PCR product and pCR®-XL-TOPO® vector were mixed gently and incubated for 5 minutes at room temperature. The 2 µl of the TOPO® cloning reaction were transformed into a vial of One Shot® TOP10 chemically competent cells, mixed gently and incubated on ice for 30 minutes. Next, the cells were then heat-shock for 30 seconds at 42°C without shaking and transferred into ice. The 250 µl of room temperature S.O.C. medium were added and the tube was shaken horizontally (250 rpm) at 37°C for 1 hour. The 50 µl from each transformant were spread on a Luria Bertani (LB) plate containing 50 µg/ml kanamycin and were further incubated overnight at 37°C. The positive colonies were collected and were cultured overnight in LB medium that containing 50 µg/ml kanamycin. Plasmids DNA were isolated from broth cultures by using the NucleoSpin® Plasmid Kit (Macherey-Nagel, Germany). The positive plasmids were confirmed the presence and correct orientation of the insert by using PCR technique.

2. Cloning of porcine Sn (N-terminal domain) and CD163 (domain 5) cDNA

The corrected size of PCR products were cut from the agarose gels. These gel bands were then purified by using Gel/PCR DNA Fragments Extraction Kit (Geneaid Company, Taiwan). The gel purified PCR products of porcine Sn (N-terminal domain) and CD163 (domain 5) were measured by using Nanodrop ND-1000 (Thermo Scientific, Wilming-Ton, DE, USA) and further cloned directly into pCR®-8-GW-TOPO® vector with pCR®-8-GW-TOPO® TA Cloning Kit (Invitrogen, USA) according to the manufacturer's instructions. Briefly, the TOPO® cloning reaction for eventual transformation into either chemically competent cells were performed in a tube of 6 µl in volume. The mixtures of fresh PCR product and pCR®-8-GW-TOPO® vector were mixed gently and incubated for 5 minutes at room temperature. The 2 µl of the TOPO® cloning reaction were transformed into a vial of One Shot® TOP10 chemically competent cells, mixed gently and incubated on ice for 30 minutes. Next, the cells were then heat-shock for 30 seconds at 42°C without shaking and transferred into ice. The 250 µl of room temperature S.O.C. medium were added and the tube was shook horizontally (250 rpm) at 37°C for 1 hour. The 50 µl from each transformant were spread on a Luria Bertani (LB) plate that containing 100 µg/ml spectinomycin and were further incubated overnight at 37°C. The positive colonies were collected and were cultured overnight in LB medium that containing 100 µg/ml spectinomycin. DNA plasmids were isolated from broth cultures by using the NucleoSpin® Plasmid Kit (Macherey-Nagel, Germany). The positive plasmids were confirmed the presence and correct orientation of the insert by using PCR technique.

3. Sequencing of porcine Sn (full and N-terminal domain) and CD163 (full and domain 5) cDNA

The positive purified recombinant plasmids were submitted to sequence at AITBIOTECH PTE LTD Company (Singapore). The sequencing primers were designed and

shown in Table 3.5. All sequences were carried out in duplicate and were determined by sequencing with both strands. Nucleotide and deduced amino acids sequences were aligned, studied the composition and compared homology by using the software of Chromas 2.33, Bioedit v7.0.5.3 program (ClustalX 2.0.11) and the MEGA 5.1 program.

Table 3.5 Nucleotide sequence primers used for sequencing

Primer	Sequence (5' to 3')	Predicted product sizes (bp)
Sequence primers of Sn (full)		
M13 reverse	CAGGAAACAGCTATGAC	650
F1 forward	GACGGAGCCGGTCAACCTACA	600
F2 forward	GGAGGGCTCTCACAGCCGCAC	600
F3 forward	CACAGATGCCGGCTCATACCA	600
F4 forward	TGCTGCCCTCTATGCTTGCCG	600
F5 forward	CACCACGGACCTGGCTGCCCC	600
F6 forward	TGTCCTCTACGCACCCCGCAG	600
F7 forward	CACTGTGGACAGCGAGCCACC	600
F8 forward	CCACCTGGGCAGTCGGCTGGT	458
Sequence primers of Sn (N-terminal domain)		
M13 forward	GTAAAACGACGGCCAGT	650
F1 forward	GACGGAGCCGGTCAACCTACA	524
Sequence primers of CD163 (full)		
M13 reverse	CAGGAAACAGCTATGAC	650
F1 forward	AGTCAAATTTCAAGAGCGGTG	600
F2 forward	GAGACTTAAAGGTGGAGGCAG	600
F3 forward	CGGCGTAGTCTGCTCAAGATA	600
F4 forward	TTTTGGGGAAGGAACAGGGCC	600
F5 forward	CTCAGCTTTGGAGGCAGGAAA	471
Sequence primers of CD163 (domain 5)		
M13 forward	GTAAAACGACGGCCAGT	541

CHAPTER IV
RESULTS

Phase I. Confirmation of PRRSV strains by using RT-PCR

Table 4.1 Confirmation of positive PRRSV strains from sera samples

Farm	Number of nursery pigs	Number of positive samples			S/P ratio ELISA
		EU	US	HP	
1	3	2	-	-	3.123
2	3	3	-	-	3.100
3	3	-	3	-	3.214
4	3	-	3	-	3.233
5	3	-	-	2	3.115
6	3	-	-	2	3.126
Total	**18**	**5**	**6**	**4**	

The author firstly selected the PRRSV infected farms with clinical signs for PRRS infection based on historical investigation of those farms. The PRRS suspected nursery pigs were selected and the sera and PAM samples were collected. The total RNA was extracted from sera samples and the ORF 1 and ORF 7 cDNA of PRRSV were amplified using RT-PCR technique. The status of PRRS serology was tested with ELISA kit. The RT-PCR results showed that US, HP and EU strains of PRRSV amplified products were detected about 756 bp, 666 bp and 478 bp, respectively (data not shown). The ELISA results also showed that the average S/P ratio of sera from PRRS suspected nursery pigs were all positive from PRRSV infection (cut-off value is 0.4). In this study, sera samples were PRRSV positive in 15/18 of the cases. Two PAM samples (1 PAM sample/farm) of each PRRSV strains were selected and used for further study.

Phase II: Expression of porcine Sn (full and N-terminal domain) and CD163 (full and domain 5) cDNA

1. The expression level of porcine Sn (full) cDNA

Six PRRSV infected PAM samples were selected from 15 positive sera samples (two infected PAM per PRRSV strain). These purified PCR products for porcine Sn cDNA with two sets of porcine Sn primers were run and stained with ethidium bromide in agarose gel 1.5 % at 100 volts for 45 minutes. The image and relative density were shown in Figure 4.1 and Figure 4.2. The product lengths for each sample were confirmed and showed at 5,193 bp. A FusionCapt Advance SL4 (Vilber Lourmat, Germany) was used to capture the gel image. The bands of Thai-HP-(1 and 2)-Sn; Thai-EU-(1 and 2)-Sn and Thai-US-(1 and 2)-Sn were analyzed the density in each lane using Bio 1D Advance software. The expression level of porcine Sn (full) cDNA was examined by RT-PCR and the density of bands. The result indicated that expression of porcine Sn (full) of PAM from US strain was higher than that of HP and EU strains.

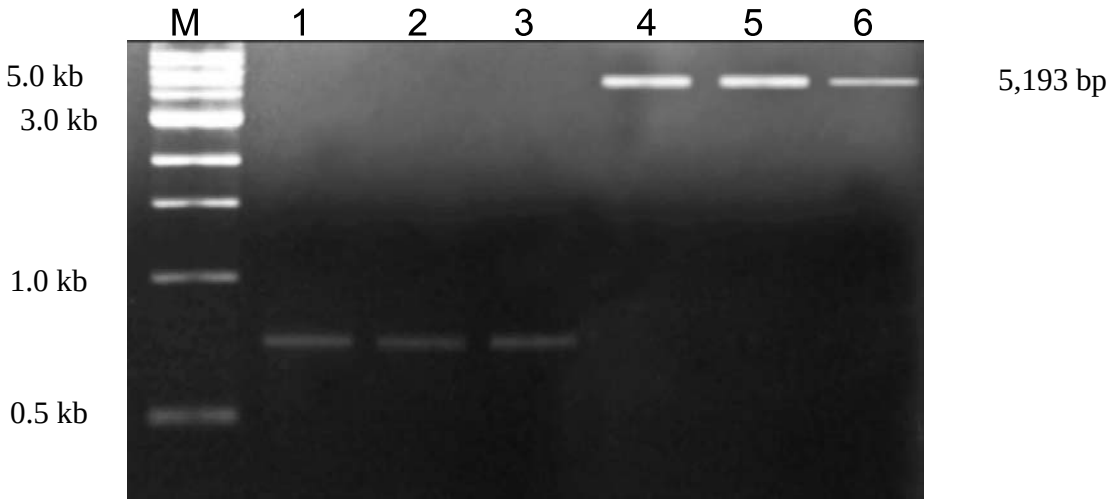

Figure 4.1 Typical 1.5 % agarose gel electrophoresis of amplification of porcine Sn (full) cDNA

Lane M, 1kb marker (BioLab, USA)

Lane 1, GAPDH from HP infected PAM

Lane 2, GAPDH from from US infected PAM

Lane 3, GAPDH from EU infected PAM

Lane 4, Thai-HP-1-Sn infected PAM

Lane 5, Thai-US-1-Sn infected PAM

Lane 6, Thai-EU-1-Sn infected PAM

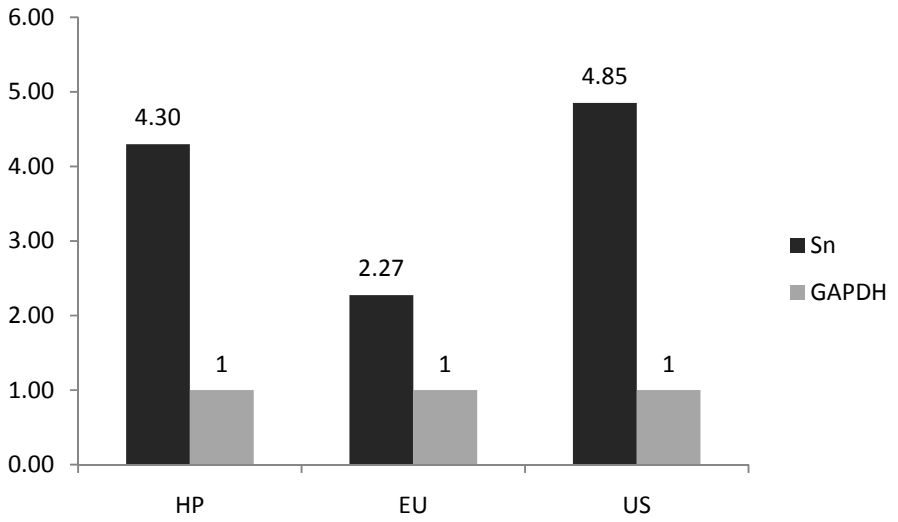

Figure 4.2 Graph shows the relative density of PCR products of porcine Sn (full) and normalized to GAPDH expression

2. The expression level of porcine Sn (N-terminal domain) cDNA

The synthesized N-terminal Sn cDNA from PRRSV infected PAM from each strain were amplified. The expected size of 1,024 bp of these genes were run and stained with ethidium bromide in agarose gel 1.5% at 100 volts for 45 minutes. A FusionCapt Advance SL4 (Vilber Lourmat, Germany) was used to capture the image. The bands of Thai-HP-N-terminal-Sn, Thai-EU-N-terminal-Sn and Thai-US-N-terminal-Sn were measured the density in each lane by using Bio 1D Advance software. The image and relative density were shown in Figure 4.3 and Figure 4.4. The expression of porcine Sn (N-terminal domain) was 6 times higher than GAPDH gene. The band of Thai-US-N-terminal-Sn was showed the highest level at the value of 8.12 followed by 7.46 and 6.35 for Thai-HP-N-terminal-Sn and Thai-EU-N-terminal-Sn, respectively. The result indicated that expression of porcine Sn (N-terminal domain) from US strain was higher than that of HP and EU strains.

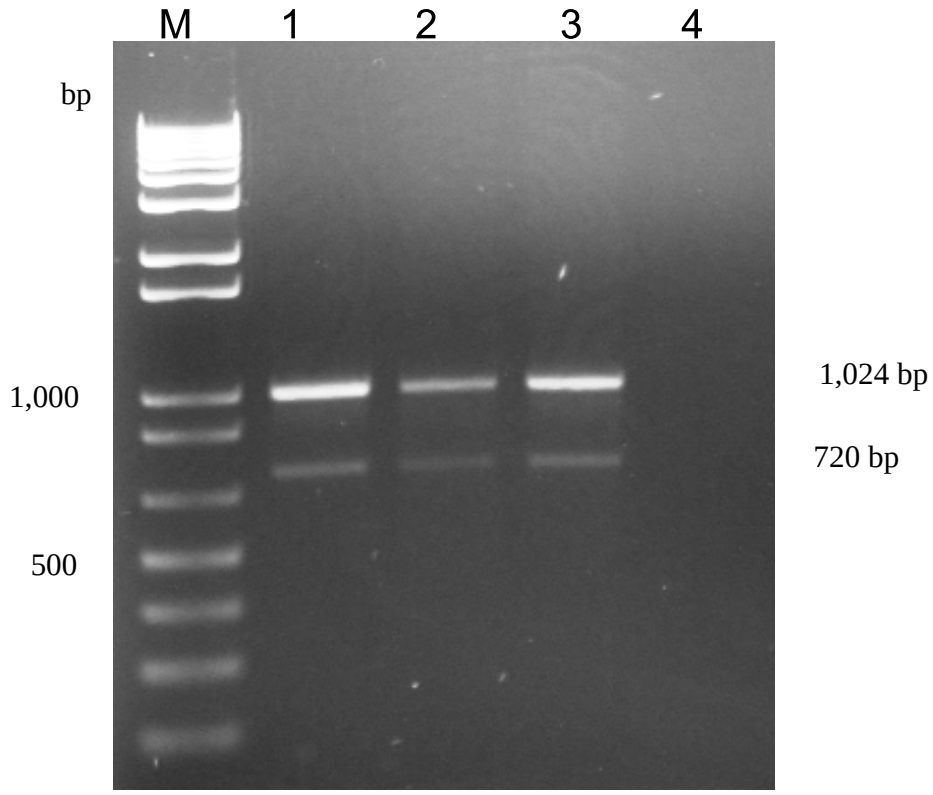

Figure 4.3 Typical 1.5 % agarose gel electrophoresis of amplification of porcine Sn (N-terminal domain) cDNA

Lane M, 1kb marker (Fermantas, Canada)

Lane 1, Thai-HP-1-N-terminal-Sn (1,024 bp); Thai-HP-1-GAPDH (720 bp)

Lane 2, Thai-EU-1-N-terminal-Sn (1,024 bp); Thai-EU-1-GAPDH (720 bp)

Lane 3, Thai-US-1-N-terminal-Sn (1,024 bp); Thai-US-1-GAPDH (720 bp)

Lane 4, negative control

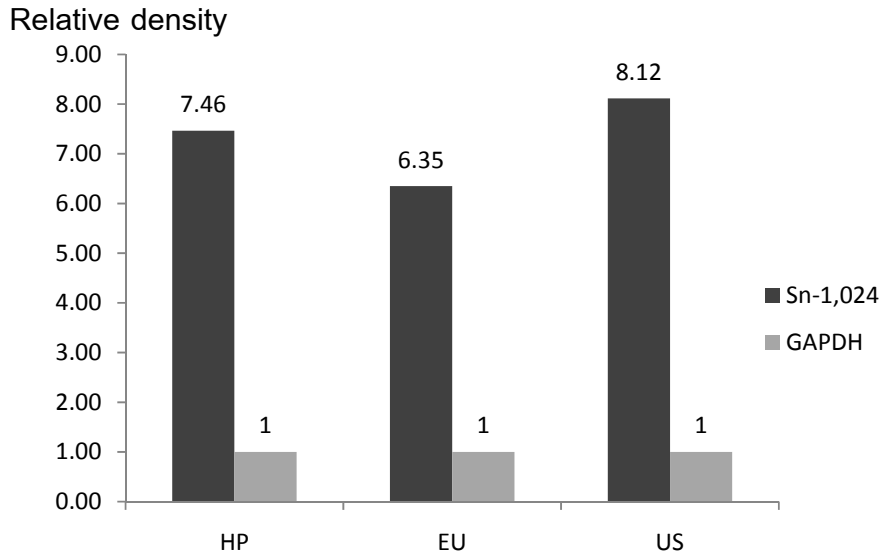

Figure 4.4 Graph shows the relative density of PCR products of porcine Sn (N-terminal domain) and normalized to GAPDH expression

3. The expression level of porcine CD163 (full) cDNA

Six PRRSV infected PAM samples were selected (two infected PAM per PRRSV strain). These purified PCR products for CD163 (full) cDNA with two pairs of porcine CD163 (full) primers were run and stained with ethidium bromide in agarose gel 1.5 % at 100 volts for 45 minutes. The PCR product lengths for each sample were confirmed and shown about 3,400 bp. A FusionCapt Advance SL4 (Vilber Lourmat, Germany) was used to capture the band image as shown in Figure 4.5. The PCR product bands from Thai-HP-CD163, Thai-EU-CD163 and Thai-US-CD163 (as shown in Figure 4.6) were analyzed the density and calculated the relative in each lane by using Bio 1D Advance software. The result indicated that expression of porcine CD163 (full) cDNA from US strain was higher than that of HP and EU strains.

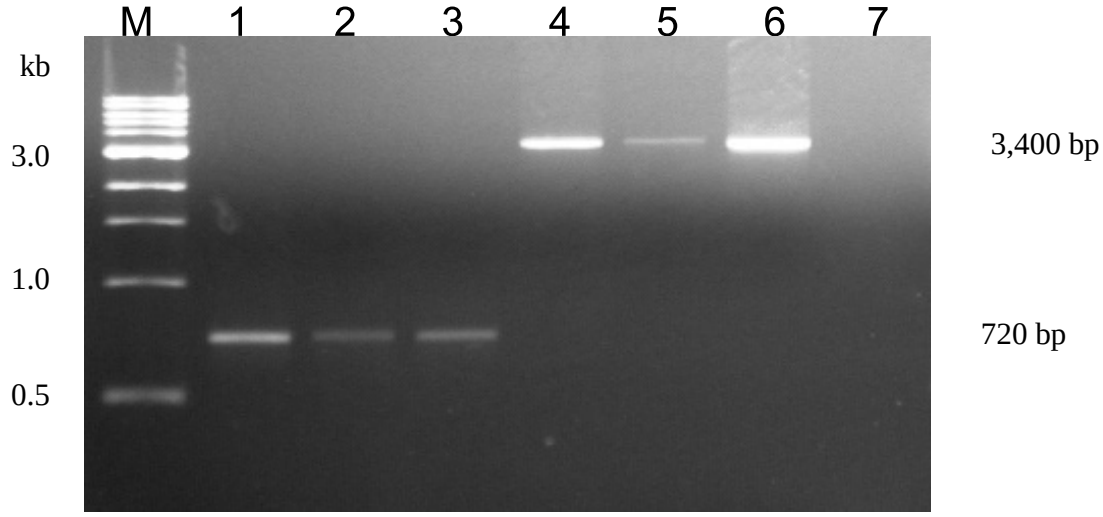

Figure 4.5 Typical 1.5 % agarose gel electrophoresis of amplification of porcine CD163 (full) cDNA

Lane M, 1kb marker (BioLab, USA)

Lane 1, GAPDH from EU infected PAM

Lane 2, GAPDH from HP infected PAM

Lane 3, GAPDH from from US infected PAM

Lane 4, Thai-HP-1-CD163 infected PAM

Lane 5, Thai-EU-1-CD163 infected PAM

Lane 6, Thai-US-1-CD163 infected PAM

Lane 7, negative control

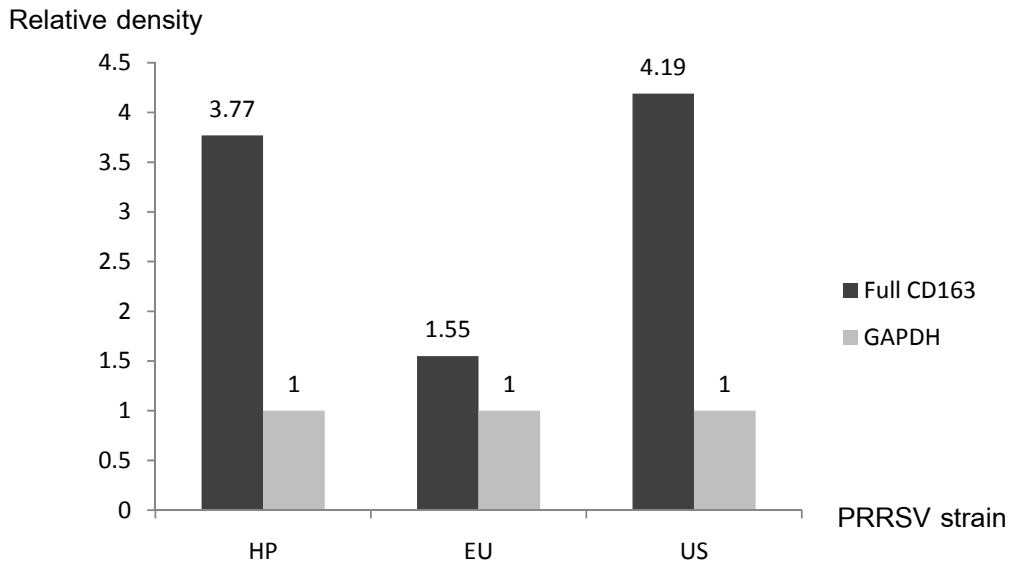

Figure 4.6 Graph shows the relative density of PCR products of porcine CD163 (full) and normalized to GAPDH expression

4. The expression level of porcine CD163 (domain 5) cDNA

The porcine CD163 (domain 5) were amplified from the cDNA of PRRSV infected PAM with specific primers, were run and were stained with ethidium bromide in agarose gel 1.5 % at 100 volts for 45 minutes. A FusionCapt Advance SL4 (Vilber Lourmat, Germany) was also used to capture the image. The expected size of PCR products were about 395 bp and the gel picture was shown in Figure 4.7. The bands of Thai-HP-CD163-DO5, Thai-EUCD163-DO5 and Thai-US-CD163-DO5 were measured the density of the bands in each lane by using Bio 1D Advance software. The relative density was shown in Figure 4.8. The result indicated that expression of porcine CD163 (domain 5) from US strain (4.71) was higher than that of HP (3.70) and EU (3.11) strains.

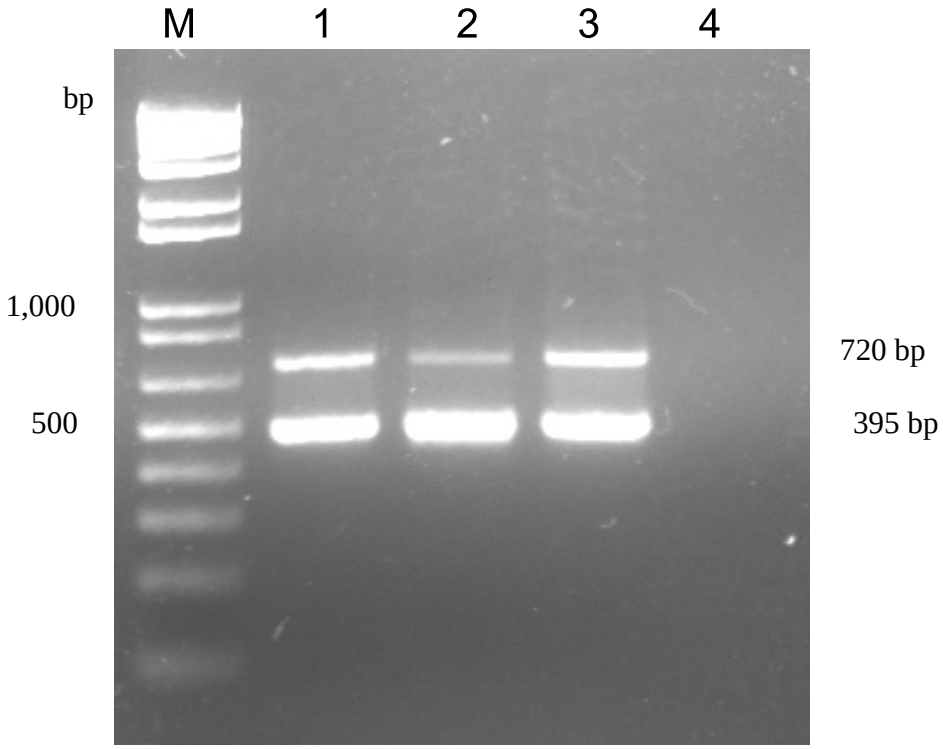

Figure 4.7 Typical 1.5 % agarose gel electrophoresis of amplification of porcine CD163 (domain 5) cDNA

Lane M, 1kb marker (Fermantas, Canada)

Lane 1, Thai-HP-1-CD163-DO5 (395 bp); Thai-HP-1-GAPDH (720 bp)

Lane 2, Thai-EU-1-CD163-DO5 (395 bp); Thai-EU-1-GAPDH (720 bp)

Lane 3, Thai-US-1-CD163-DO5 (395 bp); Thai-US-1-GAPDH (720 bp)

Lane 4, negative control

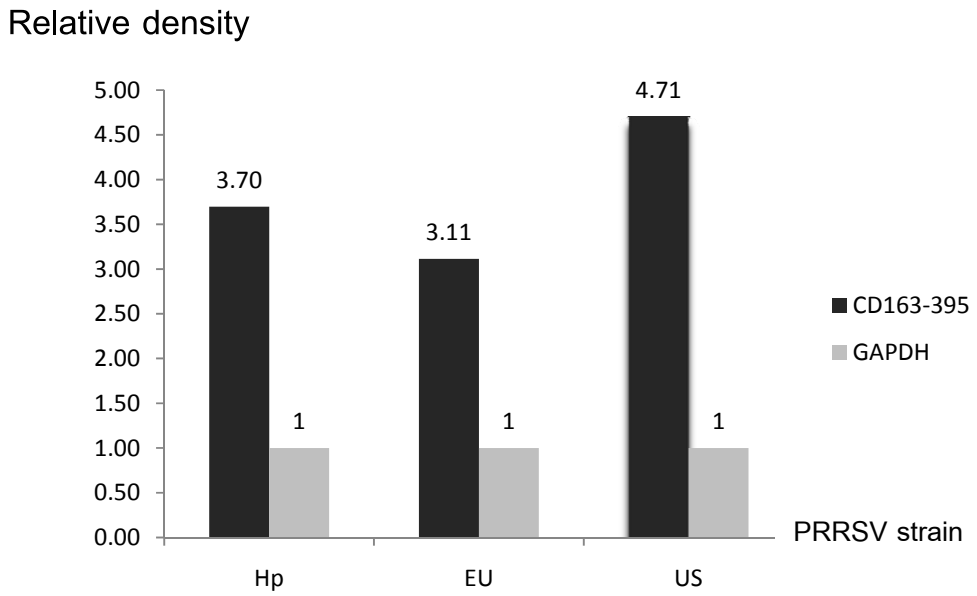

Figure 4.8 Graph shows the relative density of PCR products of porcine CD163 (domain 5) and normalized to GAPDH expression

Phase III: Cloning and sequencing of porcine Sn (full and N-terminal domain) and CD163 (full and domain 5) cDNA

1. Cloning of porcine Sn (full and N-terminal domain) and CD163 (full and domain 5) cDNA

The corrected sizes of porcine Sn (full) (5,193 bp), porcine Sn (N-terminal domain) (1,024 bp), porcine CD163 (full) (3,400 bp) and porcine CD163 (domain 5) (395 bp) cDNA from 3 strains of PRRSV infected PAM samples were cut and purified from 1.5 % agarose gel. The purified PCR products from 4 genes were first ligated into pCR®8-GW-TOPO® plasmid vectors and transformed into *E.coli* strain One Shot®TOP10 (Invitrogen, USA). Two full receptor genes showed no positive clones. Then, the author used the pCR®-XL-TOPO® plasmid vectors for further long length gene cloning. The corrected insertions of porcine Sn (full and N-terminal domain) and CD163 (full and domain 5) were confirmed the corrected

transformants by using PCR technique with specific primers. Ten out of twenty colonies of positive transformants were successfully confirmed for porcine Sn (full) cDNA and named as the clones of pCR®-XL-TOPO®-Sn (4 of Thai-US-1 to 4; 3 of Thai-EU-1 to 3 and 3 of Thai-HP-1 to 3). Twenty-four out of fifty colonies of transformants of porcine Sn (N-terminal domain) cDNA were confirmed and named as clones of pCR®8-GW-TOPO®-Sn (9 of Thai-US-1 to 9; 8 of Thai-EU-1 to 8 and 7 of Thai-HP-1 to 7). Twenty out of forty colonies of porcine CD163 (full) transformants were positive and named as pCR®-XL-TOPO®-CD163 (9 of Thai-US-1 to 9; 5 of Thai-EU-1 to 5 and 6 of Thai-HP-1 to 6) and Twenty-nine out of sixty-three colonies of transformants of porcine CD163 (domain 5) cDNA were confirmed and named as clones of pCR®8-GW-TOPO®-Sn (11 of Thai-US-1 to 11; 8 of Thai-EU-1 to 8 and 10 of Thai-HP-1 to 10). The expected sizes of PCR products for these confirmations were shown at 5,325 bp; 1,170 bp; 3,532 bp and 541 bp for recombinant porcine Sn (full and N-terminal domain) and porcine CD163 (full and domain 5), respectively. In addition, 3 of each corrected recombinant porcine Sn (full and N-terminal domain) and CD163 (full and domain 5) plasmids were further performed the sequencing.

Table 4.2 Positive transformants of recombinant porcine Sn and CD163 plasmids

Sample name	Sn		CD163	
	Full	N-terminal	Full	Domain 5
Thai-HP-1	2	3	4	4
Thai-HP-2	1	4	2	6
Thai-EU-1	1	4	3	4
Thai-EU-2	2	4	2	4
Thai-US-1	2	5	4	5
Thai-US-2	2	4	5	6
Total	**10**	**24**	**20**	**29**

2. Nucleotide and deduced amino acid sequences of recombinant porcine Sn and CD163 plasmids

Up to now, the author found that the nucleotide and deduced amino acid data of porcine Sn or porcine CD163 cDNA isolated from PRRSV infected PAM or any pig tissues were a few reports. So in this study, the data of porcine Sn and CD163 from PAM of normal pigs from GenBank databases were chosen to analyze alignment, the nucleotide and deduced amino acid compositions and the homology with the data from PAM of PRRSV infected pigs.

2.1 Nucleotide and deduced amino acid sequences of recombinant porcine Sn (full) plasmids

The author selected 3 recombinant plasmids of Thai-HP-1-pCR®-XL-TOPO®-Sn (Thai-HP-1-Sn), Thai-EU-1-pCR®-XL-TOPO®-Sn (Thai-EU-1-Sn) and Thai-US-1-pCR®-XL-TOPO®-Sn (Thai-US-1-Sn) to sequence. The complete sequences of 3 recombinant porcine Sn (full) plasmids were successfully sequenced at the same length of 5,193 bp (Table 4.3) and were analyzed and compared the alignment with the reference Sn (refSn) cDNA (accession number is NM_214346) form the PAM of normal pigs which available databases from GenBank (Figure 4.9). The results indicated that the nucleotide sequences of recombinant porcine Sn (full) plasmids were changed in 10 positions at 130; 762; 1,968; 2,289; 2,718; 2,889; 3,870; 4,295; 4,413 and 4,493, respectively.

The deduced amino acid sequences were summarized in Table 4.4 and the alignment of recombinant porcine Sn (full) plasmids from PRRVS infected PAM (1,730 amino acids) and refSn cDNA (accession number is NP_999511) from normal PAM were shown in Figure 4.10. The results showed that the amino acid sequences of 3 recombinant porcine Sn (full) plasmids were showed the similar pattern and were changed in 2 positions at 44 (Glycine to Arginine) and 1,432 (Leucine to Serine).

Table 4.3 Nucleotide sequences of 3 recombinant porcine Sn (full) plasmids

Transformant's name	Sequence
1. Thai-HP-1-Sn (5,193 bp)	atggacttcctgctcctgctcctcctcctggcttcatctgctctagcaggcctggcctcgtggac ggtttccagccccgagaccgtgcagggcatcaagggctcctgcctcatcatcccctgcacct tccgcttcccggccaacgtggaggtgccccatggcatcacagccatctggtactatgactact caggcaagcgcctggtagtgagccactccaggaacccaaaggtggtggagaaccacttc caaggccgggccctgctgttggggcaggttgaacagaggacgtgcagcctgctgctgaag gacctgcagccccaggactcgggctcctataacttccgctttgagatcagcgagggcaacc gctggtcagatgtcaaaggcacagttgtcaccgtgacagaggtgcccagcgtgcccaccat tgccttgccagccaagctgcatgagggcatggaggtggacttcaactgctccactccctatgt gtgcccgacggagccggtcaacctacagtggcaaggccaggatcccacccgctccgtca cctcccacctccagaagcttgagccctcgggcaccagccacatggagaccctgcacatgg ccctgtcctggcaggaccatggccggatcctgagctgccaggtctcagcagccgaacgca ggatgcagaaggagattcacctccaagtgcagtatgccccccaagggtgtggagatccttttc agccactccggacggaacgtccttcctggtgatctggtcaccctcagctgccaggtgaatag cagcaaccctcaggtcagttccgtgcagtgggtcaaggatgggacgaagctcaaagacca gaaacgtgtactgcagttgcgccgggcagcctgggctgatgctggcgtctacacctgccaa gccgggaatgccgtgggctcttcagtctcacccccggtcagcctccacgtcttcatggctga ggtccaggtaagccctgtgggctccatcctggagaaccagacggtgacgctggcctgcaat acacctaaggaagcgcccagcgagctgcgctacagctggtacaagaaccacgccctgct ggagggctctcacagccgcaccctccggctgcactcagttaccagggcggattcgggcttc tacttctgcgaggtgcagaacgcccggggcagagagcgctctccccctgtcagcgtggtg gtcagccacccacccctcaccccggacctaactgccttcctggagacacaggcggggctg gtgggcatcctccaatgctctgtggtcagcgagcccccagctactctggtgttgtcacacggg ggcctcatcttggcctctacctccggggagggtgaccacagcccacgcttcagtgtcgcctc

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2. Thai-EU-1-Sn (5,193 bp)	atggacttcctgctcctgctcctcctcctggcttcatctgctctagcaggcctggcctcgtggac
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                ....|....| ....|....| ....|....| ....|....| ....|....|
                     10         20         30         40         50
                  M13 Reverse
refSn           ---------- ---------- ---------- ---------- ----------
Thai-HP-1-Sn    CAGGAAACAG CTATGACCAT GATTACGCCA AGCTATTTAG GTGACGCGTT
Thai-EU-1-Sn    CAGGAAACAG CTATGACCAT GATTACGCCA AGCTATTTAG GTGACGCGTT
Thai-US-1-Sn    CAGGAAACAG CTATGACCAT GATTACGCCA AGCTATTTAG GTGACGCGTT

                ....|....| ....|....| ....|....| ....|....| ....|....|
                     60         70         80         90        100
refSn           ---------- ---------- ---------- ---------- ----------
Thai-HP-1-Sn    AGAATACTCA AGCTATGCAT CAAGCTTGGT ACCGAGCTCG GATCCACTAG
Thai-EU-1-Sn    AGAATACTCA AGCTATGCAT CAAGCTTGGT ACCGAGCTCG GATCCACTAG
Thai-US-1-Sn    AGAATACTCA AGCTATGCAT CAAGCTTGGT ACCGAGCTCG GATCCACTAG

                ....|....| ....|....| ....|....| ....|....| ....|....|
                    110        120        130        140        150
refSn           ---------- ---------- ---------- --ATGGACTT CCTGCTCCTG
Thai-HP-1-Sn    TAACGGCCGC CAGTGTGCTG GAATTCGCCC TTATGGACTT CCTGCTCCTG
Thai-EU-1-Sn    TAACGGCCGC CAGTGTGCTG GAATTCGCCC TTATGGACTT CCTGCTCCTG
Thai-US-1-Sn    TAACGGCCGC CAGTGTGCTG GAATTCGCCC TTATGGACTT CCTGCTCCTG

                ....|....| ....|....| ....|....| ....|....| ....|....|
                    160        170        180        190        200
refSn           CTCCTCCTCC TGGCTTCATC TGCTCTAGCA GGCCTGGCCT CGTGGACGGT
Thai-HP-1-Sn    CTCCTCCTCC TGGCTTCATC TGCTCTAGCA GGCCTGGCCT CGTGGACGGT
Thai-EU-1-Sn    CTCCTCCTCC TGGCTTCATC TGCTCTAGCA GGCCTGGCCT CGTGGACGGT
Thai-US-1-Sn    CTCCTCCTCC TGGCTTCATC TGCTCTAGCA GGCCTGGCCT CGTGGACGGT

                ....|....| ....|....| ....|....| ....|....| ....|....|
                    210        220        230        240        250
refSn           TTCCAGCCCC GAGACCGTGC AGGGCATCAA GGGCTCCTGC CTCATCATCC
Thai-HP-1-Sn    TTCCAGCCCC GAGACCGTGC AGGGCATCAA GGGCTCCTGC CTCATCATCC
Thai-EU-1-Sn    TTCCAGCCCC GAGACCGTGC AGGGCATCAA GGGCTCCTGC CTCATCATCC
Thai-US-1-Sn    TTCCAGCCCC GAGACCGTGC AGGGCATCAA GGGCTCCTGC CTCATCATCC

                ....|....| ....|....| ....|....| ....|....| ....|....|
                    260        270        280        290        300
refSn           CCTGCACCTT GGGCTTCCCG GCCAACGTGG AGGTGCCCCA TGGCATCACA
Thai-HP-1-Sn    CCTGCACCTT CCGCTTCCCG GCCAACGTGG AGGTGCCCCA TGGCATCACA
Thai-EU-1-Sn    CCTGCACCTT GGGCTTCCCG GCCAACGTGG AGGTGCCCCA TGGCATCACA
Thai-US-1-Sn    CCTGCACCTT CCGCTTCCCG GCCAACGTGG AGGTGCCCCA TGGCATCACA

                ....|....| ....|....| ....|....| ....|....| ....|....|
                    310        320        330        340        350
refSn           GCCATCTGGT ACTATGACTA CTCAGGCAAG CGCCTGGTAG TGAGCCACTC
Thai-HP-1-Sn    GCCATCTGGT ACTATGACTA CTCAGGCAAG CGCCTGGTAG TGAGCCACTC
Thai-EU-1-Sn    GCCATCTGGT ACTATGACTA CTCAGGCAAG CGCCTGGTAG TGAGCCACTC
Thai-US-1-Sn    GCCATCTGGT ACTATGACTA CTCAGGCAAG CGCCTGGTAG TGAGCCACTC

                ....|....| ....|....| ....|....| ....|....| ....|....|
                    360        370        380        390        400
refSn           CAGGAACCCA AAGGTGGTGG AGAACCACTT CCAAGGCCGG GCCCTGCTGT
Thai-HP-1-Sn    CAGGAACCCA AAGGTGGTGG AGAACCACTT CCAAGGCCGG GCCCTGCTGT
Thai-EU-1-Sn    CAGGAACCCA AAGGTGGTGG AGAACCACTT CCAAGGCCGG GCCCTGCTGT
Thai-US-1-Sn    CAGGAACCCA AAGGTGGTGG AGAACCACTT CCAAGGCCGG GCCCTGCTGT
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                ....|....| ....|....| ....|....| ....|....| ....|....|
                       410        420        430        440        450
refSn           TGGGGCAGGT TGAACAGAGG ACGTGCAGCC TGCTGCTGAA GGACCTGCAG
Thai-HP-1-Sn    TGGGGCAGGT TGAACAGAGG ACGTGCAGCC TGCTGCTGAA GGACCTGCAG
Thai-EU-1-Sn    TGGGGCAGGT TGAACAGAGG ACGTGCAGCC TGCTGCTGAA GGACCTGCAG
Thai-US-1-Sn    TGGGGCAGGT TGAACAGAGG ACGTGCAGCC TGCTGCTGAA GGACCTGCAG

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       460        470        480        490        500
refSn           CCCCAGGACT CGGGCTCCTA TAACTTCCGC TTTGAGATCA GCGAGGGCAA
Thai-HP-1-Sn    CCCCAGGACT CGGGCTCCTA TAACTTCCGC TTTGAGATCA GCGAGGGCAA
Thai-EU-1-Sn    CCCCAGGACT CGGGCTCCTA TAACTTCCGC TTTGAGATCA GCGAGGGCAA
Thai-US-1-Sn    CCCCAGGACT CGGGCTCCTA TAACTTCCGC TTTGAGATCA GCGAGGGCAA

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       510        520        530        540        550
refSn           CCGCTGGTCA GATGTCAAAG GCACAGTTGT CACCGTGACA GAGGTGCCCA
Thai-HP-1-Sn    CCGCTGGTCA GATGTCAAAG GCACAGTTGT CACCGTGACA GAGGTGCCCA
Thai-EU-1-Sn    CCGCTGGTCA GATGTCAAAG GCACAGTTGT CACCGTGACA GAGGTGCCCA
Thai-US-1-Sn    CCGCTGGTCA GATGTCAAAG GCACAGTTGT CACCGTGACA GAGGTGCCCA

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       560        570        580        590        600
refSn           GCGTGCCCAC CATTGCCTTG CCAGCCAAGC TGCATGAGGG CATGGAGGTG
Thai-HP-1-Sn    GCGTGCCCAC CATTGCCTTG CCAGCCAAGC TGCATGAGGG CATGGAGGTG
Thai-EU-1-Sn    GCGTGCCCAC CATTGCCTTG CCAGCCAAGC TGCATGAGGG CATGGAGGTG
Thai-US-1-Sn    GCGTGCCCAC CATTGCCTTG CCAGCCAAGC TGCATGAGGG CATGGAGGTG

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       610        620        630        640        650
refSn           GACTTCAACT GCTCCACTCC CTATGTGTGC CCGACGGAGC CGGTCAACCT
Thai-HP-1-Sn    GACTTCAACT GCTCCACTCC CTATGTGTGC CCGACGGAGC CGGTCAACCT
Thai-EU-1-Sn    GACTTCAACT GCTCCACTCC CTATGTGTGC CCGACGGAGC CGGTCAACCT
Thai-US-1-Sn    GACTTCAACT GCTCCACTCC CTATGTGTGC CCGACGGAGC CGGTCAACCT

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       660        670        680        690        700
refSn           ACAGTGGCAA GGCCAGGATC CCACCCGCTC CGTCACCTCC CACCTCCAGA
Thai-HP-1-Sn    ACAGTGGCAA GGCCAGGATC CCACCCGCTC CGTCACCTCC CACCTCCAGA
Thai-EU-1-Sn    ACAGTGGCAA GGCCAGGATC CCACCCGCTC CGTCACCTCC CACCTCCAGA
Thai-US-1-Sn    ACAGTGGCAA GGCCAGGATC CCACCCGCTC CGTCACCTCC CACCTCCAGA

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       710        720        730        740        750
refSn           AGCTTGAGCC CTCGGGCACC AGCCACATGG AGACCCTGCA CATGGCCCTG
Thai-HP-1-Sn    AGCTTGAGCC CTCGGGCACC AGCCACATGG AGACCCTGCA CATGGCCCTG
Thai-EU-1-Sn    AGCTTGAGCC CTCGGGCACC AGCCACATGG AGACCCTGCA CATGGCCCTG
Thai-US-1-Sn    AGCTTGAGCC CTCGGGCACC AGCCACATGG AGACCCTGCA CATGGCCCTG

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       760        770        780        790        800
refSn           TCCTGGCAGG ACCATGGCCG GATCCTGAGC TGCCAGGTCT CAGCAGCCGA
Thai-HP-1-Sn    TCCTGGCAGG ACCATGGCCG GATCCTGAGC TGCCAGGTCT CAGCAGCCGA
Thai-EU-1-Sn    TCCTGGCAGG ACCATGGCCG GATCCTGAGC TGCCAGGTCT CAGCAGCCGA
Thai-US-1-Sn    TCCTGGCAGG ACCATGGCCG GATCCTGAGC TGCCAGGTCT CAGCAGCCGA
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                ....|....| ....|....| ....|....| ....|....| ....|....|
                        810        820        830        840        850
refSn           ACGCAGGATG CAGAAGGAGA TTCACCTCCA AGTGCAGTAT GCCCCCAAGG
Thai-HP-1-Sn    ACGCAGGATG CAGAAGGAGA TTCACCTCCA AGTGCAGTAT GCCCCCAAGG
Thai-EU-1-Sn    ACGCAGGATG CAGAAGGAGA TTCACCTCCA AGTGCAGTAT GCCCCCAAGG
Thai-US-1-Sn    ACGCAGGATG CAGAAGGAGA TTCACCTCCA AGTGCAGTAT GCCCCCAAGG

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        860        870        880        890        900
refSn           GTGTGGAGAT CCTTTTCAGC CACTCCGGAC GGAACGTCCT TCCAGGTGAT
Thai-HP-1-Sn    GTGTGGAGAT CCTTTTCAGC CACTCCGGAC GGAACGTCCT TCGTGGTGAT
Thai-EU-1-Sn    GTGTGGAGAT CCTTTTCAGC CACTCCGGAC GGAACGTCCT TCCAGGTGAT
Thai-US-1-Sn    GTGTGGAGAT CCTTTTCAGC CACTCCGGAC GGAACGTCCT TCGTGGTGAT

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        910        920        930        940        950
refSn           CTGGTCACCC TCAGCTGCCA GGTGAATAGC AGCAACCCTC AGGTCAGTTC
Thai-HP-1-Sn    CTGGTCACCC TCAGCTGCCA GGTGAATAGC AGCAACCCTC AGGTCAGTTC
Thai-EU-1-Sn    CTGGTCACCC TCAGCTGCCA GGTGAATAGC AGCAACCCTC AGGTCAGTTC
Thai-US-1-Sn    CTGGTCACCC TCAGCTGCCA GGTGAATAGC AGCAACCCTC AGGTCAGTTC

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       960        970        980        990       1000
refSn           CGTGCAGTGG GTCAAGGATG GGACGAAGCT CAAAGACCAG AAACGTGTAC
Thai-HP-1-Sn    CGTGCAGTGG GTCAAGGATG GGACGAAGCT CAAAGACCAG AAACGTGTAC
Thai-EU-1-Sn    CGTGCAGTGG GTCAAGGATG GGACGAAGCT CAAAGACCAG AAACGTGTAC
Thai-US-1-Sn    CGTGCAGTGG GTCAAGGATG GGACGAAGCT CAAAGACCAG AAACGTGTAC

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        1010       1020       1030       1040       1050
refSn           TGCAGTTGCG CCGGGCAGCC TGGGCTGATG CTGGCGTCTA CACCTGCCAA
Thai-HP-1-Sn    TGCAGTTGCG CCGGGCAGCC TGGGCTGATG CTGGCGTCTA CACCTGCCAA
Thai-EU-1-Sn    TGCAGTTGCG CCGGGCAGCC TGGGCTGATG CTGGCGTCTA CACCTGCCAA
Thai-US-1-Sn    TGCAGTTGCG CCGGGCAGCC TGGGCTGATG CTGGCGTCTA CACCTGCCAA

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                       1060       1070       1080       1090       1100
refSn           GCCGGGAATG CCGTGGGCTC TTCAGTCTCA CCCCCGGTCA GCCTCCACGT
Thai-HP-1-Sn    GCCGGGAATG CCGTGGGCTC TTCAGTCTCA CCCCCGGTCA GCCTCCACGT
Thai-EU-1-Sn    GCCGGGAATG CCGTGGGCTC TTCAGTCTCA CCCCCGGTCA GCCTCCACGT
Thai-US-1-Sn    GCCGGGAATG CCGTGGGCTC TTCAGTCTCA CCCCCGGTCA GCCTCCACGT

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                        1110       1120       1130       1140       1150
refSn           CTTCATGGCT GAGGTCCAGG TAAGCCCTGT GGGCTCCATC CTGGAGAACC
Thai-HP-1-Sn    CTTCATGGCT GAGGTCCAGG TAAGCCCTGT GGGCTCCATC CTGGAGAACC
Thai-EU-1-Sn    CTTCATGGCT GAGGTCCAGG TAAGCCCTGT GGGCTCCATC CTGGAGAACC
Thai-US-1-Sn    CTTCATGGCT GAGGTCCAGG TAAGCCCTGT GGGCTCCATC CTGGAGAACC

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                        1160       1170       1180       1190       1200
refSn           AGACGGTGAC GCTGGCCTGC AATACACCTA AGGAAGCGCC CAGCGAGCTG
Thai-HP-1-Sn    AGACGGTGAC GCTGGCCTGC AATACACCTA AGGAAGCGCC CAGCGAGCTG
Thai-EU-1-Sn    AGACGGTGAC GCTGGCCTGC AATACACCTA AGGAAGCGCC CAGCGAGCTG
Thai-US-1-Sn    AGACGGTGAC GCTGGCCTGC AATACACCTA AGGAAGCGCC CAGCGAGCTG
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                        1210       1220       1230       1240       1250
refSn           CGCTACAGCT GGTACAAGAA CCACGCCCTG CTGGAGGGCT CTCACAGCCG
Thai-HP-1-Sn    CGCTACAGCT GGTACAAGAA CCACGCCCTG CTGGAGGGCT CTCACAGCCG
Thai-EU-1-Sn    CGCTACAGCT GGTACAAGAA CCACGCCCTG CTGGAGGGCT CTCACAGCCG
Thai-US-1-Sn    CGCTACAGCT GGTACAAGAA CCACGCCCTG CTGGAGGGCT CTCACAGCCG

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                        1260       1270       1280       1290       1300
refSn           CACCCTCCGG CTGCACTCAG TTACCAGGGC GGATTCGGGC TTCTACTTCT
Thai-HP-1-Sn    CACCCTCCGG CTGCACTCAG TTACCAGGGC GGATTCGGGC TTCTACTTCT
Thai-EU-1-Sn    CACCCTCCGG CTGCACTCAG TTACCAGGGC GGATTCGGGC TTCTACTTCT
Thai-US-1-Sn    CACCCTCCGG CTGCACTCAG TTACCAGGGC GGATTCGGGC TTCTACTTCT

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                        1310       1320       1330       1340       1350
refSn           GCGAGGTGCA GAACGCCCGG GGCAGAGAGC GCTCTCCCCC TGTCAGCGTG
Thai-HP-1-Sn    GCGAGGTGCA GAACGCCCGG GGCAGAGAGC GCTCTCCCCC TGTCAGCGTG
Thai-EU-1-Sn    GCGAGGTGCA GAACGCCCGG GGCAGAGAGC GCTCTCCCCC TGTCAGCGTG
Thai-US-1-Sn    GCGAGGTGCA GAACGCCCGG GGCAGAGAGC GCTCTCCCCC TGTCAGCGTG

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                        1360       1370       1380       1390       1400
refSn           GTGGTCAGCC ACCCACCCCT CACCCCGGAC CTAACTGCCT TCCTGGAGAC
Thai-HP-1-Sn    GTGGTCAGCC ACCCACCCCT CACCCCGGAC CTAACTGCCT TCCTGGAGAC
Thai-EU-1-Sn    GTGGTCAGCC ACCCACCCCT CACCCCGGAC CTAACTGCCT TCCTGGAGAC
Thai-US-1-Sn    GTGGTCAGCC ACCCACCCCT CACCCCGGAC CTAACTGCCT TCCTGGAGAC

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                        1410       1420       1430       1440       1450
refSn           ACAGGCGGGG CTGGTGGGCA TCCTCCAATG CTCTGTGGTC AGCGAGCCCC
Thai-HP-1-Sn    ACAGGCGGGG CTGGTGGGCA TCCTCCAATG CTCTGTGGTC AGCGAGCCCC
Thai-EU-1-Sn    ACAGGCGGGG CTGGTGGGCA TCCTCCAATG CTCTGTGGTC AGCGAGCCCC
Thai-US-1-Sn    ACAGGCGGGG CTGGTGGGCA TCCTCCAATG CTCTGTGGTC AGCGAGCCCC

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                        1460       1470       1480       1490       1500
refSn           CAGCTACTCT GGTGTTGTCA CACGGGGGCC TCATCTTGGC CTCTACCTCC
Thai-HP-1-Sn    CAGCTACTCT GGTGTTGTCA CACGGGGGCC TCATCTTGGC CTCTACCTCC
Thai-EU-1-Sn    CAGCTACTCT GGTGTTGTCA CACGGGGGCC TCATCTTGGC CTCTACCTCC
Thai-US-1-Sn    CAGCTACTCT GGTGTTGTCA CACGGGGGCC TCATCTTGGC CTCTACCTCC

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                        1510       1520       1530       1540       1550
refSn           GGGGAGGGTG ACCACAGCCC ACGCTTCAGT GTCGCCTCTG CCCCCAACTC
Thai-HP-1-Sn    GGGGAGGGTG ACCACAGCCC ACGCTTCAGT GTCGCCTCTG CCCCCAACTC
Thai-EU-1-Sn    GGGGAGGGTG ACCACAGCCC ACGCTTCAGT GTCGCCTCTG CCCCCAACTC
Thai-US-1-Sn    GGGGAGGGTG ACCACAGCCC ACGCTTCAGT GTCGCCTCTG CCCCCAACTC

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                        1560       1570       1580       1590       1600
refSn           CCTGCGCCTG GAGATTCAAG ACCTGGGGCC AACAGACAGT GGGGAATACA
Thai-HP-1-Sn    CCTGCGCCTG GAGATTCAAG ACCTGGGGCC AACAGACAGT GGGGAATACA
Thai-EU-1-Sn    CCTGCGCCTG GAGATTCAAG ACCTGGGGCC AACAGACAGT GGGGAATACA
Thai-US-1-Sn    CCTGCGCCTG GAGATTCAAG ACCTGGGGCC AACAGACAGT GGGGAATACA
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                        1610       1620       1630       1640       1650
refSn           TGTGCTCAGC CAGCAGTTCT CTTGGGAATG CGTCCTCCAC CCTGGACTTC
Thai-HP-1-Sn    TGTGCTCAGC CAGCAGTTCT CTTGGGAATG CGTCCTCCAC CCTGGACTTC
Thai-EU-1-Sn    TGTGCTCAGC CAGCAGTTCT CTTGGGAATG CGTCCTCCAC CCTGGACTTC
Thai-US-1-Sn    TGTGCTCAGC CAGCAGTTCT CTTGGGAATG CGTCCTCCAC CCTGGACTTC

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                        1660       1670       1680       1690       1700
refSn           CATGCCAATG CAGCCCGCCT CCTCATCAGC CCAGCAGCAG AGGTGGTGGA
Thai-HP-1-Sn    CATGCCAATG CAGCCCGCCT CCTCATCAGC CCAGCAGCAG AGGTGGTGGA
Thai-EU-1-Sn    CATGCCAATG CAGCCCGCCT CCTCATCAGC CCAGCAGCAG AGGTGGTGGA
Thai-US-1-Sn    CATGCCAATG CAGCCCGCCT CCTCATCAGC CCAGCAGCAG AGGTGGTGGA

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                        1710       1720       1730       1740       1750
refSn           AGGGCAGGCG GTGACACTGA GCTGCAGGAG CAGCCTGAGC CTGATGCCTG
Thai-HP-1-Sn    AGGGCAGGCG GTGACACTGA GCTGCAGGAG CAGCCTGAGC CTGATGCCTG
Thai-EU-1-Sn    AGGGCAGGCG GTGACACTGA GCTGCAGGAG CAGCCTGAGC CTGATGCCTG
Thai-US-1-Sn    AGGGCAGGCG GTGACACTGA GCTGCAGGAG CAGCCTGAGC CTGATGCCTG

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                        1760       1770       1780       1790       1800
refSn           ACACCCGTTT TTCCTGGTAC CTGAACGGGG CCCTGATTCT CGAGGGGCCC
Thai-HP-1-Sn    ACACCCGTTT TTCCTGGTAC CTGAACGGGG CCCTGATTCT CGAGGGGCCC
Thai-EU-1-Sn    ACACCCGTTT TTCCTGGTAC CTGAACGGGG CCCTGATTCT CGAGGGGCCC
Thai-US-1-Sn    ACACCCGTTT TTCCTGGTAC CTGAACGGGG CCCTGATTCT CGAGGGGCCC

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                        1810       1820       1830       1840       1850
refSn           AGCAGCAGCC TCCTGCTCCC AGCAGCCTCC AGCACAGATG CCGGCTCATA
Thai-HP-1-Sn    AGCAGCAGCC TCCTGCTCCC AGCAGCCTCC AGCACAGATG CCGGCTCATA
Thai-EU-1-Sn    AGCAGCAGCC TCCTGCTCCC AGCAGCCTCC AGCACAGATG CCGGCTCATA
Thai-US-1-Sn    AGCAGCAGCC TCCTGCTCCC AGCAGCCTCC AGCACAGATG CCGGCTCATA

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                        1860       1870       1880       1890       1900
refSn           CCACTGCCGG GCCCAGAACA GCCACAGCAC CAGCGGGCCC TCCTCACCTG
Thai-HP-1-Sn    CCACTGCCGG GCCCAGAACA GCCACAGCAC CAGCGGGCCC TCCTCACCTG
Thai-EU-1-Sn    CCACTGCCGG GCCCAGAACA GCCACAGCAC CAGCGGGCCC TCCTCACCTG
Thai-US-1-Sn    CCACTGCCGG GCCCAGAACA GCCACAGCAC CAGCGGGCCC TCCTCACCTG

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                        1910       1920       1930       1940       1950
refSn           CTGTTCTCAC CGTGCTCTAC GCCCCACGCC AGCCCGTGTT CACTGCCCAG
Thai-HP-1-Sn    CTGTTCTCAC CGTGCTCTAC GCCCCACGCC AGCCCGTGTT CACTGCCCAG
Thai-EU-1-Sn    CTGTTCTCAC CGTGCTCTAC GCCCCACGCC AGCCCGTGTT CACTGCCCAG
Thai-US-1-Sn    CTGTTCTCAC CGTGCTCTAC GCCCCACGCC AGCCCGTGTT CACTGCCCAG

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                        1960       1970       1980       1990       2000
refSn           CTGGACCCTG ATACTGCAGG AGCTGGGGCC GGACGCCAAG GCCTCCTCTT
Thai-HP-1-Sn    CTGGACCCTG ATACTGCAGG AGCTGGGGCC GGACGCCAAG GCCTCCTCTT
Thai-EU-1-Sn    CTGGACCCTG ATACTGCAGG AGCTGGGGCC GGACGCCAAG GCCTCCTCTT
Thai-US-1-Sn    CTGGACCCTG ATACTGCAGG AGCTGGGGCC GGACGCCAAG GCCTCCTCTT
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                        2010       2020       2030       2040       2050
refSn           GTGCCGTGTG GACAGCGACC CCCCAGCCCA GCTGCAGCTG CTCCACAGGG
Thai-HP-1-Sn    GTGCCGTGTG GACAGCGACC CCCCAGCCCA GCTGCAGCTG CTCCACAGGG
Thai-EU-1-Sn    GTGCCGTGTG GACAGCGACC CCCCAGCCCA GCTGCAGCTG CTCCACAGGG
Thai-US-1-Sn    GTGCCGTGTG GACAGCGACC CCCCAGCCCA GCTGCAGCTG CTCCACAGGG

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                        2060       2070       2080       2090       2100
refSn           GCCGTGTTGT GGCCTCTTCT CTGTCATGGG GGGGCGGCTG CTGCACCTGC
Thai-HP-1-Sn    GCCGTGTTGT GGCCTCTTCT CTGTCATGGG GGGGCGGCTG CTGCACCTGC
Thai-EU-1-Sn    GCCGTGTTGT GGCCTCTTCT CTGTCATGGG GGGGCGGCTG CTGCACCTGT
Thai-US-1-Sn    GCCGTGTTGT GGCCTCTTCT CTGTCATGGG GGGGCGGCTG CTGCACCTGT

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                        2110       2120       2130       2140       2150
refSn           GGAGGCTGTT TCCACCGCAT GAAGGTCACC AAAGCACCCA ACCTACTGCG
Thai-HP-1-Sn    GGAGGCTGTT TCCACCGCAT GAAGGTCACC AAAGCACCCA ACCTACTGCG
Thai-EU-1-Sn    GGAGGCTGTT TCCACCGCAT GAAGGTCACC AAAGCACCCA ACCTACTGCG
Thai-US-1-Sn    GGAGGCTGTT TCCACCGCAT GAAGGTCACC AAAGCACCCA ACCTACTGCG

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                        2160       2170       2180       2190       2200
refSn           TGTAGAGATC CGAGACCCGG TGCTGGAGGA TGAGGGTGTG TACCTGTGCG
Thai-HP-1-Sn    TGTAGAGATC CGAGACCCGG TGCTGGAGGA TGAGGGTGTG TACCTGTGCG
Thai-EU-1-Sn    TGTAGAGATC CGAGACCCGG TGCTGGAGGA TGAGGGTGTG TACCTGTGCG
Thai-US-1-Sn    TGTAGAGATC CGAGACCCGG TGCTGGAGGA TGAGGGTGTG TACCTGTGCG

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                        2210       2220       2230       2240       2250
refSn           AGGCCAGCAG CGCCCTGGGC AACGCCTCCG CCTCTGCAAC CTTGGATGCC
Thai-HP-1-Sn    AGGCCAGCAG CGCCCTGGGC AACGCCTCCG CCTCTGCAAC CTTGGATGCC
Thai-EU-1-Sn    AGGCCAGCAG CGCCCTGGGC AACGCCTCCG CCTCTGCAAC CTTGGATGCC
Thai-US-1-Sn    AGGCCAGCAG CGCCCTGGGC AACGCCTCCG CCTCTGCAAC CTTGGATGCC

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                        2260       2270       2280       2290       2300
refSn           CAGGCCACTG TCCTGGTCAT CACACCGTCA CACACGCTGC AGGAAGGCAT
Thai-HP-1-Sn    CAGGCCACTG TCCTGGTCAT CACACCGTCA CACACGCTGC AGGAAGGCAT
Thai-EU-1-Sn    CAGGCCACTG TCCTGGTCAT CACACCGTCA CACACGCTGC AGGAAGGCAT
Thai-US-1-Sn    CAGGCCACTG TCCTGGTCAT CACACCGTCA CACACGCTGC AGGAAGGCAT

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                        2310       2320       2330       2340       2350
refSn           TGAAGCCAAC CTGACTTGCA ACGTGAGCCG TGAAGCCAGC GGCCCTGCCA
Thai-HP-1-Sn    TGAAGCCAAC CTGACTTGCA ACGTGAGCCG TGAAGCCAGC GGCCCTGCCA
Thai-EU-1-Sn    TGAAGCCAAC CTGACTTGCA ACGTGAGCCG TGAAGCCAGC GGCCCTGCCA
Thai-US-1-Sn    TGAAGCCAAC CTGACTTGCA ACGTGAGCCG TGAAGCCAGC GGCCCTGCCA

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                        2360       2370       2380       2390       2400
refSn           ACTTCTCCTG GTTCCGAGAT GGGGCGCTAT GGGCCCAGGG CCCTCTGGAC
Thai-HP-1-Sn    ACTTCTCCTG GTTCCGAGAT GGGGCGCTAT GGGCCCAGGG CCCTCTGGAC
Thai-EU-1-Sn    ACTTCTCCTG GTTCCGAGAT GGGGCGCTAT GGGCCCAGGG CCCTCTGGAC
Thai-US-1-Sn    ACTTCTCCTG GTTCCGAGAT GGGGCGCTAT GGGCCCAGGG CCCTCTGGAC
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                       2410       2420       2430       2440       2450
refSn           ACCGTGACGC TGCTACCTGT GGCCAGAACT GATGCTGCCC TCTATGCTTG
Thai-HP-1-Sn    ACCGTGACGC TGCTACCTGT AGCCAGAACT GATGCTGCCC TCTATGCTTG
Thai-EU-1-Sn    ACCGTGACGC TGCTACCTGT AGCCAGAACT GATGCTGCCC TCTATGCTTG
Thai-US-1-Sn    ACCGTGACGC TGCTACCTGT AGCCAGAACT GATGCTGCCC TCTATGCTTG

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                       2460       2470       2480       2490       2500
refSn           CCGCATCGTC ACCGAGGCTG GTGCTGGCCT CTCCACCCCT GTGGCCCTGA
Thai-HP-1-Sn    CCGCATCGTC ACCGAGGCTG GTGCTGGCCT CTCCACCCCT GTGGCCCTGA
Thai-EU-1-Sn    CCGCATCGTC ACCGAGGCTG GTGCTGGCCT CTCCACCCCT GTGGCCCTGA
Thai-US-1-Sn    CCGCATCGTC ACCGAGGCTG GTGCTGGCCT CTCCACCCCT GTGGCCCTGA

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                       2510       2520       2530       2540       2550
refSn           ATGTGCTCTA TCCCCCCGAT CCTCCAAAGT TGTCAGCCCT CCTGGACGTG
Thai-HP-1-Sn    ATGTGCTCTA TCCCCCCGAT CCTCCAAAGT TGTCAGCCCT CCTGGACGTG
Thai-EU-1-Sn    ATGTGCTCTA TCCCCCCGAT CCTCCAAAGT TGTCAGCCCT CCTGGACGTG
Thai-US-1-Sn    ATGTGCTCTA TCCCCCCGAT CCTCCAAAGT TGTCAGCCCT CCTGGACGTG

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                       2560       2570       2580       2590       2600
refSn           GACCAGGGCC ACACGGCTGT GTTCGTCTGT ACTGTGGACA GTCGCCCTCT
Thai-HP-1-Sn    GACCAGGGCC ACACGGCTGT GTTCGTCTGT ACTGTGGACA GTCGCCCTCT
Thai-EU-1-Sn    GACCAGGGCC ACACGGCTGT GTTCGTCTGT ACTGTGGACA GTCGCCCTCT
Thai-US-1-Sn    GACCAGGGCC ACACGGCTGT GTTCGTCTGT ACTGTGGACA GTCGCCCTCT

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                       2610       2620       2630       2640       2650
refSn           TGCCCAGTTG GCCCTGTTCC GTGGGGAACA CCTCCTGGCC GCCAGCTCGG
Thai-HP-1-Sn    TGCCCAGTTG GCCCTGTTCC GTGGGGAACA CCTCCTGGCC GCCAGCTCGG
Thai-EU-1-Sn    TGCCCAGTTG GCCCTGTTCC GTGGGGAACA CCTCCTGGCC GCCAGCTCGG
Thai-US-1-Sn    TGCCCAGTTG GCCCTGTTCC GTGGGGAACA CCTCCTGGCC GCCAGCTCGG

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                       2660       2670       2680       2690       2700
refSn           CACTCCGGCT CCCCCCTCGT GGCCGCCTCC AGGCCAAAGC CTCGGCCAAC
Thai-HP-1-Sn    CACTCCGGCT CCCCCCTCGT GGCCGCCTCC AGGCCAAAGC CTCGGCCAAC
Thai-EU-1-Sn    CACTCCGGCT CCCCCCTCGT GGCCGCCTCC AGGCCAAAGC CTCGGCCAAC
Thai-US-1-Sn    CACTCCGGCT CCCCCCTCGT GGCCGCCTCC AGGCCAAAGC CTCGGCCAAC

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                       2710       2720       2730       2740       2750
refSn           TCCTTGCAGC TAGAGGTCCG AGACTTGAGC CTTGGGGACT CTGGCAGCTA
Thai-HP-1-Sn    TCCTTGCAGC TAGAGGTCCG AGACTTGAGC CTTGGGGACT CTGGCAGCTA
Thai-EU-1-Sn    TCCTTGCAGC TAGAGGTCCG AGACTTGAGC CTTGGGGACT CTGGCAGCTA
Thai-US-1-Sn    TCCTTGCAGC TAGAGGTCCG AGACTTGAGC CTTGGGGACT CTGGCAGCTA

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                       2760       2770       2780       2790       2800
refSn           CCACTGTGAG GCCACCAACA TCCTTGGATC AGCCAACACT TCTCTTACCT
Thai-HP-1-Sn    CCACTGTGAG GCCACCAACA TCCTTGGATC AGCCAACACT TCTCTTACCT
Thai-EU-1-Sn    CCACTGTGAG GCCACCAACA TCCTTGGATC AGCCAACACT TCTCTTACCT
Thai-US-1-Sn    CCACTGTGAG GCCACCAACA TCCTTGGATC AGCCAACACT TCTCTTACCT
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                       2810       2820       2830       2840       2850
refSn           TCCAGGTCCG AGGAGCCTGG GTCCGGGTGT CACCGTCGCC TGAGCTCCAG
Thai-HP-1-Sn    TCCAGGTCCG AGGAGCCTGG GTCCGGGTGT CACCGTCGCC TGAGCTCCAG
Thai-EU-1-Sn    TCCAGGTCCG AGGAGCCTGG GTCCGGGTGT CACCGTCGCC TGAGCTCCAA
Thai-US-1-Sn    TCCAGGTCCG AGGAGCCTGG GTCCGGGTGT CACCGTCGCC TGAGCTCCAG

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                      2860       2870       2880       2890       2900
refSn           GAGGGCCAGG CTGTGGTCCT GAGCTGCCAG GTACCCATAG GGGTCCTGGA
Thai-HP-1-Sn    GAGGGCCAGG CTGTGGTCCT GAGCTGCCAG GTACCCATAG GGGTCCTGGA
Thai-EU-1-Sn    GAGGGCCAGG CTGTGGTCCT GAGCTGCCAG GTACCCATAG GGGTCCTGGA
Thai-US-1-Sn    GAGGGCCAGG CTGTGGTCCT GAGCTGCCAG GTACCCATAG GGGTCCTGGA

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                       2910       2920       2930       2940       2950
refSn           GGGGACCTCA TATCGTTGGT ATCGGGATGG CCAGCCCCTC CAGGAGTCCA
Thai-HP-1-Sn    GGGGACCTCA TATCGTTGGT ATCGGGATGG CCAGCCCCTC CAGGAGTCCA
Thai-EU-1-Sn    GGGGACCTCA TATCGTTGGT ATCGGGATGG CCAGCCCCTC CAGGAGTCCA
Thai-US-1-Sn    GGGGACCTCA TATCGTTGGT ATCGGGATGG CCAGCCCCTC CAGGAGTCCA

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                       2960       2970       2980       2990       3000
refSn           CTTCGGCCAC GCTCCGTTTT GCAGCCATAA CTCTGAGCCA GGCTGGAGCC
Thai-HP-1-Sn    CTTCGGCCAC GCTCCGTTTT GCAGCCATAA CTCTGAGCCA GGCTGGAGCC
Thai-EU-1-Sn    CTTCGGCCAC GCTCCGTTTT GCAGCCATAA CTCTGAGCCA GGCTGGAGCC
Thai-US-1-Sn    CTTCGGCCAC GCTCCGTTTT GCAGCCATAA CTCTGAGCCA GGCTGGAGCC

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                       3010       3020       3030       3040       3050
refSn           TACCATTGCC AAGCCCAAGC TCCAGGCTCA GCCACCACGG ACCTGGCTGC
Thai-HP-1-Sn    TACCATTGCC AAGCCCAAGC GCCAGGCTCA GCCACCACGG ACCTGGCTGC
Thai-EU-1-Sn    TACCATTGCC AAGCCCAAGC GCCAGGCTCA GCCACCACGG ACCTGGCTGC
Thai-US-1-Sn    TACCATTGCC AAGCCCAAGC GCCAGGCTCA GCCACCACGG ACCTGGCTGC

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                       3060       3070       3080       3090       3100
refSn           CCCTGTCAGC CTCCACGTGA CCTACGCACC TCGCCAGGCC ACACTCACCA
Thai-HP-1-Sn    CCCTGTCAGC CTCCACGTGA CCTACGCACC TCGCCAGGCC ACACTCACCA
Thai-EU-1-Sn    CCCTGTCAGC CTCCACGTGA CCTACGCACC TCGCCAGGCC ACACTCACCA
Thai-US-1-Sn    CCCTGTCAGC CTCCACGTGA CCTACGCACC TCGCCAGGCC ACACTCACCA

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                       3110       3120       3130       3140       3150
refSn           CCCTGATGGA CTCAGGCCTC GGGCGACTGG GCCTCCTTCT GTGCCGTGTG
Thai-HP-1-Sn    CCCTGATGGA CTCAGGCCTC GGGCGACTGG GCCTCCTTCT GTGCCGTGTG
Thai-EU-1-Sn    CCCTGATGGA CTCAGGCCTC GGGCGACTGG GCCTCCTTCT GTGCCGTGTG
Thai-US-1-Sn    CCCTGATGGA CTCAGGCCTC GGGCGACTGG GCCTCCTTCT GTGCCGTGTG

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                       3160       3170       3180       3190       3200
refSn           AACAGTGACC CTCCTGCCCA GCTCCGACTG CTCCATGGGA GCCGCCTCGT
Thai-HP-1-Sn    AACAGTGACC CTCCTGCCCA GCTCCGACTG CTCCATGGGA GCCGCCTCGT
Thai-EU-1-Sn    AACAGTGACC CTCCTGCCCA GCTCCGACTG CTCCATGGGA GCCGCCTCGT
Thai-US-1-Sn    AACAGTGACC CTCCTGCCCA GCTCCGACTG CTCCATGGGA GCCGCCTCGT
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                        3210       3220       3230       3240       3250
refSn           GGCCTCTACT CTACAAGGTG TGGAGGAGCT TGCAGGCAGC TCTCCCCGCC
Thai-HP-1-Sn    GGCCTCTACT CTACAAGGTG TGGAGGAGCT TGCAGGCAGC TCTCCCCGCC
Thai-EU-1-Sn    GGCCTCTACT CTACAAGGTG TGGAGGAGCT TGCAGGCAGC TCTCCCCGCC
Thai-US-1-Sn    GGCCTCTACT CTACAAGGTG TGGAGGAGCT TGCAGGCAGC TCTCCCCGCC

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                        3260       3270       3280       3290       3300
refSn           TACAGGTGGC CACAGCCCCC AACACGCTGC GCCTGGAGAT CCACAACGCA
Thai-HP-1-Sn    TACAGGTGGC CACAGCCCCC AACACGCTGC GCCTGGAGAT CCACAACGCA
Thai-EU-1-Sn    TACAGGTGGC CACAGCCCCC AACACGCTGC GCCTGGAGAT CCACAACGCA
Thai-US-1-Sn    TACAGGTGGC CACAGCCCCC AACACGCTGC GCCTGGAGAT CCACAACGCA

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                        3310       3320       3330       3340       3350
refSn           GTGCTGGAGG ATGAAGGCGT CTACACCTGC GAGGCCACCA ACACCCTGGG
Thai-HP-1-Sn    GTGCTGGAGG ATGAAGGCGT CTACACCTGC GAGGCCACCA ACACCCTGGG
Thai-EU-1-Sn    GTGCTGGAGG ATGAAGGCGT CTACACCTGC GAGGCCACCA ACACCCTGGG
Thai-US-1-Sn    GTGCTGGAGG ATGAAGGCGT CTACACCTGC GAGGCCACCA ACACCCTGGG

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                        3360       3370       3380       3390       3400
refSn           TCAGACCTTG GCCTCCGCCG CCTTCGATGC CCAGGCTATG AGAGTGCAGG
Thai-HP-1-Sn    TCAGACCTTG GCCTCCGCCG CCTTCGATGC CCAGGCTATG AGAGTGCAGG
Thai-EU-1-Sn    TCAGACCTTG GCCTCCGCCG CCTTCGATGC CCAGGCTATG AGAGTGCAGG
Thai-US-1-Sn    TCAGACCTTG GCCTCCGCCG CCTTCGATGC CCAGGCTATG AGAGTGCAGG

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                        3410       3420       3430       3440       3450
refSn           TGTGGCCCAA TGCCACCGTG CAAGAGGGGC AGCTGGTGAA CCTGACCTGC
Thai-HP-1-Sn    TGTGGCCCAA TGCCACCGTG CAAGAGGGGC AGCTGGTGAA CCTGACCTGC
Thai-EU-1-Sn    TGTGGCCCAA TGCCACCGTG CAAGAGGGGC AGCTGGTGAA CCTGACCTGC
Thai-US-1-Sn    TGTGGCCCAA TGCCACCGTG CAAGAGGGGC AGCTGGTGAA CCTGACCTGC

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                        3460       3470       3480       3490       3500
refSn           CTTGTATGGA CCACGCACCT GGCCCAGCTC ACCTACACGT GGTACCGAGA
Thai-HP-1-Sn    CTTGTATGGA CCACGCACCT GGCCCAGCTC ACCTACACGT GGTACCGAGA
Thai-EU-1-Sn    CTTGTATGGA CCACGCACCT GGCCCAGCTC ACCTACACGT GGTACCGAGA
Thai-US-1-Sn    CTTGTATGGA CCACGCACCT GGCCCAGCTC ACCTACACGT GGTACCGAGA

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                        3510       3520       3530       3540       3550
refSn           CCAGCAGCAG CTCCCAGGTG CTGCCCACTC CATCCTCCTG CCCAATGTCA
Thai-HP-1-Sn    CCAGCAGCAG CTCCCAGGTG CTGCCCACTC CATCCTCCTG CCCAATGTCA
Thai-EU-1-Sn    CCAGCAGCAG CTCCCAGGTG CTGCCCACTC CATCCTCCTG CCCAATGTCA
Thai-US-1-Sn    CCAGCAGCAG CTCCCAGGTG CTGCCCACTC CATCCTCCTG CCCAATGTCA

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                        3560       3570       3580       3590       3600
refSn           CTGTCACAGA TGCCGCCTCC TACCGCTGTG GCATATTGAT CCCTGGCCAG
Thai-HP-1-Sn    CTGTCACAGA TGCCGCCTCC TACCGCTGTG GCATATTGAT CCCTGGCCAG
Thai-EU-1-Sn    CTGTCACAGA TGCCGCCTCC TACCGCTGTG GCATATTGAT CCCTGGCCAG
Thai-US-1-Sn    CTGTCACAGA TGCCGCCTCC TACCGCTGTG GCATATTGAT CCCTGGCCAG
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                ....|....| ....|....| ....|....| ....|....| ....|....|
                        3610       3620       3630       3640       3650
refSn           GCACTCCGCC TCTCCAGACC TGTCGCCCTG GATGTCCTCT ACGCACCCCG
Thai-HP-1-Sn    GCACTCCGCC TCTCCAGACC TGTCGCCCTG GATGTCCTCT ACGCACCCCG
Thai-EU-1-Sn    GCACTCCGCC TCTCCAGACC TGTCGCCCTG GATGTCCTCT ACGCACCCCG
Thai-US-1-Sn    GCACTCCGCC TCTCCAGACC TGTCGCCCTG GATGTCCTCT ACGCACCCCG

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                        3660       3670       3680       3690       3700
refSn           CAGACTGCGC CTGACCCATC TCTTGGAGAG CCGTGGTGGG CAGCTGGCCG
Thai-HP-1-Sn    CAGACTGCGC CTGACCCATC TCTTGGAGAG CCGTGGTGGG CAGCTGGCCG
Thai-EU-1-Sn    CAGACTGCGC CTGACCCATC TCTTGGAGAG CCGTGGTGGG CAGCTGGCCG
Thai-US-1-Sn    CAGACTGCGC CTGACCCATC TCTTGGAGAG CCGTGGTGGG CAGCTGGCCG

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                        3710       3720       3730       3740       3750
refSn           TGGTGCTGTG CACTGTGGAC AGTCGCCCAG CTGCCCAGCT GACCCTCAGC
Thai-HP-1-Sn    TGGTGCTGTG CACTGTGGAC AGTCGCCCAG CTGCCCAGCT GACCCTCAGC
Thai-EU-1-Sn    TGGTGCTGTG CACTGTGGAC AGTCGCCCAG CTGCCCAGCT GACCCTCAGC
Thai-US-1-Sn    TGGTGCTGTG CACTGTGGAC AGTCGCCCAG CTGCCCAGCT GACCCTCAGC

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                        3760       3770       3780       3790       3800
refSn           CATGCTGGCC GCCTCCTGGC CTCCTCAACC GCAGCCTCTG TCCCCAACAC
Thai-HP-1-Sn    CATGCTGGCC GCCTCCTGGC CTCCTCAACC GCAGCCTCTG TCCCCAACAC
Thai-EU-1-Sn    CATGCTGGCC GCCTCCTGGC CTCCTCAACC GCAGCCTCTG TCCCCAACAC
Thai-US-1-Sn    CATGCTGGCC GCCTCCTGGC CTCCTCAACC GCAGCCTCTG TCCCCAACAC

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                        3810       3820       3830       3840       3850
refSn           CCTGCGCCTG GAGCTGTGGG AGCCCCGGCC CAGTGATGAG GGTCTCTACA
Thai-HP-1-Sn    CCTGCGCCTG GAGCTGTGGG AGCCCCGGCC CAGTGATGAG GGTCTCTACA
Thai-EU-1-Sn    CCTGCGCCTG GAGCTGTGGG AGCCCCGGCC CAGTGATGAG GGTCTCTACA
Thai-US-1-Sn    CCTGCGCCTG GAGCTGTGGG AGCCCCGGCC CAGTGATGAG GGTCTCTACA

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                        3860       3870       3880       3890       3900
refSn           GCTGCTCGGC CCGCAGTCCT CTGGGCCAGG CCAACACATC CCTGGAGCTG
Thai-HP-1-Sn    GCTGCTCGGC CCGCAGTCCT CTGGGCCAGG CCAACACATC CCTGGAGCTG
Thai-EU-1-Sn    GCTGCTCGGC CCGCAGTCCT CTGGGCCAGG CCAACACATC CCTGGAGCTG
Thai-US-1-Sn    GCTGCTCGGC CCGCAGTCCT CTGGGCCAGG CCAACACATC CCTGGAGCTG

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                        3910       3920       3930       3940       3950
refSn           CGGCTAGAGG GCGTGCAGGT GGCACTGGCT CCATCGGCCA CTGTGCCGGA
Thai-HP-1-Sn    CGGCTAGAGG GCGTGCAGGT GGCACTGGCT CCATCGGCCA CTGTGCCGGA
Thai-EU-1-Sn    CGGCTAGAGG GCGTGCAGGT GGCACTGGCT CCATCGGCCA CTGTGCCGGA
Thai-US-1-Sn    CGGCTAGAGG GCGTGCAGGT GGCACTGGCT CCATCGGCCA CTGTGCCGGA

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                        3960       3970       3980       3990       4000
refSn           GGGGGCCCCT GTCACAGTGA CCTGTGAAGA CCCTGCTGCC CGCCCACCCA
Thai-HP-1-Sn    GGGGGCCCCT GTCACAGTGA CCTGTGAAGA CCCTGCTGCC CGCCCACCCA
Thai-EU-1-Sn    GGGGGCCCCT GTCACAGTGA CCTGTGAAGA CCCTGCTGCC CGCCCACCCA
Thai-US-1-Sn    GGGGGCCCCT GTCACAGTGA CCTGTGAAGA CCCTGCTGCC CGCCCACCCA
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                ....|....| ....|....| ....|....| ....|....| ....|....|
                        4010       4020       4030       4040       4050
refSn           CTCTCTATGT CTGGTACCAC AACAGCCGTT GGCTGCAGGA GGGGTCGGCT
Thai-HP-1-Sn    CCCTCTATGT CTGGTACCAC AACAGCCGTT GGCTGCAGGA GGGGTCGGCT
Thai-EU-1-Sn    CCCTCTATGT CTGGTACCAC AACAGCCGTT GGCTGCAGGA GGGGTCGGCT
Thai-US-1-Sn    CCCTCTATGT CTGGTACCAC AACAGCCGTT GGCTGCAGGA GGGGTCGGCT

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                        4060       4070       4080       4090       4100
refSn           GCCTCCCTCT CGTTTCCAGC GGCTACACGG GCTCACGCGG GCGCCTATAC
Thai-HP-1-Sn    GCCTCCCTCT CGTTTCCAGC GGCTACACGG GCTCACGCGG GCGCCTATAC
Thai-EU-1-Sn    GCCTCCCTCT CGTTTCCAGC GGCTACACGG GCTCACGCGG GCGCCTATAC
Thai-US-1-Sn    GCCTCCCTCT CGTTTCCAGC GGCTACACGG GCTCACGCGG GCGCCTATAC

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                        4110       4120       4130       4140       4150
refSn           CTGCCAGGTC CAGGATGCCC AGGGCACACG CATCTCCCAG CCCGCAGCAC
Thai-HP-1-Sn    CTGCCAGGTC CAGGATGCCC AGGGCACACG CATCTCCCAG CCCGCAGCAC
Thai-EU-1-Sn    CTGCCAGGTC CAGGATGCCC AGGGCACACG CATCTCCCAG CCCGCAGCAC
Thai-US-1-Sn    CTGCCAGGTC CAGGATGCCC AGGGCACACG CATCTCCCAG CCCGCAGCAC

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                        4160       4170       4180       4190       4200
refSn           TGCACATCCT CTATGCCCCT CGGGATGCTG TCCTTTCCTC CTTCTGGGAC
Thai-HP-1-Sn    TGCACATCCT CTATGCCCCT CGGGATGCTG TCCTTTCCTC CTTCTGGGAC
Thai-EU-1-Sn    TGCACATCCT CTATGCCCCT CGGGATGCTG TCCTTTCCTC CTTCTGGGAC
Thai-US-1-Sn    TGCACATCCT CTATGCCCCT CGGGATGCTG TCCTTTCCTC CTTCTGGGAC

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                        4210       4220       4230       4240       4250
refSn           TCAAGGGCCA GCCCTATGGC CGTGGTACAG TGCACTGTGG ACAGCGAGCC
Thai-HP-1-Sn    TCAAGGGCCA GCCCTATGGC CGTGGTACAG TGCACTGTGG ACAGCGAGCC
Thai-EU-1-Sn    TCAAGGGCCA GCCCTATGGC CGTGGTACAG TGCACTGTGG ACAGCGAGCC
Thai-US-1-Sn    TCAAGGGCCA GCCCTATGGC CGTGGTACAG TGCACTGTGG ACAGCGAGCC

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                        4260       4270       4280       4290       4300
refSn           ACCTGCCGAG ATGACCCTGT CCCATGATGG CAAGGTGCTG GCCACCAGCC
Thai-HP-1-Sn    ACCTGCCGAG ATGACCCTGT CCCATGATGG CAAGGTGCTG GCCACCAGCC
Thai-EU-1-Sn    ACCTGCCGAG ATGACCCTGT CCCATGATGG CAAGGTGCTG GCCACCAGCC
Thai-US-1-Sn    ACCTGCCGAG ATGACCCTGT CCCATGATGG CAAGGTGCTG GCCACCAGCC

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                        4310       4320       4330       4340       4350
refSn           ATGGGGTCCA CGGCTTAGCA GTGGGGACAG GCCATGTCCA GGTGGCCCGC
Thai-HP-1-Sn    ATGGGGTCCA CGGCTTAGCA GTGGGGACAG GCCATGTCCA GGTGGCCCGC
Thai-EU-1-Sn    ATGGGGTCCA CGGCTTAGCA GTGGGGACAG GCCATGTCCA GGTGGCCCGC
Thai-US-1-Sn    ATGGGGTCCA CGGCTTAGCA GTGGGGACAG GCCATGTCCA GGTGGCCCGC

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                        4360       4370       4380       4390       4400
refSn           AACGCCCTGC AGCTGCGGGT GCAGAATGTG CCCTCACGTG ACAAGGACAC
Thai-HP-1-Sn    AACGCCCTGC AGCTGCGGGT GCAGAATGTG CCCTCACGTG ACAAGGACAC
Thai-EU-1-Sn    AACGCCCTGC AGCTGCGGGT GCAGAATGTG CCCTCACGTG ACAAGGACAC
Thai-US-1-Sn    AACGCCCTGC AGCTGCGGGT GCAGAATGTG CCCTCACGTG ACAAGGACAC
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                       4410       4420       4430       4440       4450
refSn           CTACGTCTGC ATGGACCGCA ACTCCTTGGG CTCAGTCAGC ACCATGGGGC
Thai-HP-1-Sn    CTACGTCTGC ATGGACCGCA ACTCCTCGGG CTCAGTCAGC ACCATGGGGC
Thai-EU-1-Sn    CTACGTCTGC ATGGACCGCA ACTCCTTGGG CTCAGTCAGC ACCATGGGGC
Thai-US-1-Sn    CTACGTCTGC ATGGACCGCA ACTCCTCGGG CTCAGTCAGC ACCATGGGGC

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                       4460       4470       4480       4490       4500
refSn           AGCTGCAGCC AGAAGGTGTG CACGTGGTAG CTGAGCCAGG GCTGGATGTG
Thai-HP-1-Sn    AGCTGCAGCC AGAAGGTGTG CACGTGGTAG CTGAGCCAGG GCTGGATGTG
Thai-EU-1-Sn    AGCTGCAGCC AGAAGGTGTG CACGTGGTAG CTGAGCCAGG GCTGGATGTG
Thai-US-1-Sn    AGCTGCAGCC AGAAGGTGTG CACGTGGTAG CTGAGCCAGG GCTGGATGTG

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                       4510       4520       4530       4540       4550
refSn           CCTGAAGGCA CAGCGCTGAA CCTGAGCTGT CGCCTCCCTA GTGGCCCTGG
Thai-HP-1-Sn    CCTGAAGGCA CAGCGCTGAA CCTGAGCTGT CGCCTCCCTA GTGGCCCTGG
Thai-EU-1-Sn    CCTGAAGGCA CAGCGCTGAA CCTGAGCTGT CGCCTCCCTA GTGGTCCTGG
Thai-US-1-Sn    CCTGAAGGCA CAGCGCTGAA CCTGAGCTGT CGCCTCCCTA GTGGCCCTGG

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                       4560       4570       4580       4590       4600
refSn           GCACATAGGC AACTCCACCT TTGCTTGGTT CCGGAACGGT CGGCAGCTAC
Thai-HP-1-Sn    GCACATAGGC AACTCCACCT TTGCTTGGTT CCGGAACGGT CGGCAGCTAC
Thai-EU-1-Sn    GCACATAGGC AACTCCACCT TTGCTTGGTT CCGGAACGGT CGGCAGCTAC
Thai-US-1-Sn    GCACATAGGC AACTCCACCT TTGCTTGGTT CCGGAACGGT CGGCAGCTAC

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                       4610       4620       4630       4640       4650
refSn           ACACAGAGTC TGTGCCCACC CTTACCTTCA CCCATGTGGC CCGCGCCCAA
Thai-HP-1-Sn    ACACAGAGTC TGTGCCCACT CTTACCTTCA CCCATGTGGC CCGCGCCCAA
Thai-EU-1-Sn    ACACAGAGTC TGTGCCCACT CTTACCTTCA CCCATGTGGC CCGCGCCCAA
Thai-US-1-Sn    ACACAGAGTC TGTGCCCACT CTTACCTTCA CCCATGTGGC CCGCGCCCAA

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                       4660       4670       4680       4690       4700
refSn           GCTGGCTTGT ACCACTGCCA GGCTGAGCTC CCCGCCGGGG CTGCCACCTC
Thai-HP-1-Sn    GCTGGCTTGT ACCACTGCCA GGCTGAGCTC CCCGCCGGGG CTGCCACCTC
Thai-EU-1-Sn    GCTGGCTTGT ACCACTGCCA GGCTGAGCTC CCCGCCGGGG CTGCCACCTC
Thai-US-1-Sn    GCTGGCTTGT ACCACTGCCA GGCTGAGCTC CCCGCCGGGG CTGCCACCTC

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                       4710       4720       4730       4740       4750
refSn           TGCTCCAGTC TTGCTCCGGG TGCTCTACCC TCCCAAGACG CCCACCATGA
Thai-HP-1-Sn    TGCTCCAGTC TTGCTCCGGG TGCTCTACCC TCCCAAGACG CCCACCATGA
Thai-EU-1-Sn    TGCTCCAGTC TTGCTCCGGG TGCTCTACCC TCCCAAGACG CCCACCATGA
Thai-US-1-Sn    TGCTCCAGTC TTGCTCCGGG TGCTCTACCC TCCCAAGACG CCCACCATGA

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                       4760       4770       4780       4790       4800
refSn           CTGTTTTTGT GGAGCCCGAG GGTGGCATCC AGGGCATTCT GGACTGCCGA
Thai-HP-1-Sn    CTGTTTTTGT GGAGCCCGAG GGTGGCATCC AGGGCATTCT GGACTGCCGA
Thai-EU-1-Sn    CTGTTTTTGT GGAGCCCGAG GGTGGCATCC AGGGCATTCT GGACTGCCGA
Thai-US-1-Sn    CTGTTTTTGT GGAGCCCGAG GGTGGCATCC AGGGCATTCT GGACTGCCGA
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                        4810       4820       4830       4840       4850
refSn           GTGGACAGTG AGCCCCTAGC CAGCCTGACC CTCCACCTGG GCAGTCGGCT
Thai-HP-1-Sn    GTGGACAGTG AGCCCCTAGC CAGCCTGACC CTCCACCTGG GCAGTCGGCT
Thai-EU-1-Sn    GTGGACAGTG AGCCCCTAGC CAGCCTGACC CTCCACCTGG GCAGTCGGCT
Thai-US-1-Sn    GTGGACAGTG AGCCCCTAGC CAGCCTGACC CTCCACCTGG GCAGTCGGCT

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                        4860       4870       4880       4890       4900
refSn           GGTGGCCTCC AGCCAGCCTC AGGCTGCCCC TGCCAAGCCG CACATCCGCG
Thai-HP-1-Sn    GGTGGCCTCC AGCCAGCCTC AGGCTGCCCC TGCCAAGCCG CACATCCGCG
Thai-EU-1-Sn    GGTGGCCTCC AGCCAGCCTC AGGCTGCCCC TGCCAAGCCG CACATCCGCG
Thai-US-1-Sn    GGTGGCCTCC AGCCAGCCTC AGGCTGCCCC TGCCAAGCCG CACATCCGCG

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                        4910       4920       4930       4940       4950
refSn           TCTCAGCCAG TCCCAATGCC TTGCGAGTGG ACATGGAGGA GCTGAAGCCC
Thai-HP-1-Sn    TCTCAGCCAG TCCCAATGCC TTGCGAGTGG ACATGGAGGA GCTGAAGCCC
Thai-EU-1-Sn    TCTCAGCCAG TCCCAATGCC TTGCGAGTGG ACATGGAGGA GCTGAAGCCC
Thai-US-1-Sn    TCTCAGCCAG TCCCAATGCC TTGCGAGTGG ACATGGAGGA GCTGAAGCCC

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                        4960       4970       4980       4990       5000
refSn           AGTGACCAGG GGGAGTATGT GTGCTCGGCC TCCAATGCCC TGGGCTCTGC
Thai-HP-1-Sn    AGTGACCAGG GGGAGTATGT GTGCTCGGCC TCCAATGCCC TGGGCTCTGC
Thai-EU-1-Sn    AGTGACCAGG GGGAGTATGT GTGCTCGGCC TCCAATGCCC TGGGCTCTGC
Thai-US-1-Sn    AGTGACCAGG GGGAGTATGT GTGCTCGGCC TCCAATGCCC TGGGCTCTGC

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                        5010       5020       5030       5040       5050
refSn           CTCTGCTGCC ACCTACTTCG GAACCAGAGC CCTGCATCGC CTGCATCTGT
Thai-HP-1-Sn    CTCTGCTGCC ACCTACTTCG GAACCAGAGC CCTGCATCGC CTGCATCTGT
Thai-EU-1-Sn    CTCTGCTGCC ACCTACTTCG GAACCAGAGC CCTGCATCGC CTGCATCTGT
Thai-US-1-Sn    CTCTGCTGCC ACCTACTTCG GAACCAGAGC CCTGCATCGC CTGCATCTGT

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                        5060       5070       5080       5090       5100
refSn           TCCAGCACCT TCTCTGGTTC CTGGGGCTGC TGGCGAGCCT CCTCTTCCTA
Thai-HP-1-Sn    TCCAGCACCT TCTCTGGTTC CTGGGGCTGC TGGCGAGCCT CCTCTTCCTA
Thai-EU-1-Sn    TCCAGCACCT TCTCTGGTTC CTGGGGCTGC TGGCGAGCCT CCTCTTCCTA
Thai-US-1-Sn    TCCAGCACCT TCTCTGGTTC CTGGGGCTGC TGGCGAGCCT CCTCTTCCTA

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                        5110       5120       5130       5140       5150
refSn           CTGTTGGGCC TGGGGGTCTG GTACGCCTGG AGACGGGGAA ATTTTTACAA
Thai-HP-1-Sn    CTGTTGGGCC TGGGGGTCTG GTACGCCTGG AGACGGGGCA ATTTTTACAA
Thai-EU-1-Sn    CTGTTGGGCC TGGGGGTCTG GTACGCCTGG AGACGGGGAA ATTTTTACAA
Thai-US-1-Sn    CTGTTGGGCC TGGGGGTCTG GTACGCCTGG AGACGGGGGA ATTTTTACAA

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                        5160       5170       5180       5190       5200
refSn           GCTGAGAATG GGCGAATATT CAGTAGAGAT GGTATCTCGG AAGGAAACCA
Thai-HP-1-Sn    GCTGAGAATG GGCGAATATT CAGTAGAGAT GGTATCTCGG AAGGAAACCA
Thai-EU-1-Sn    GCTGAGAATG GGCGAATATT CAGTAGAGAT GGTATCTCGG AAGGAAACCA
Thai-US-1-Sn    GCTGAGAATG GGCGAATATT CAGTAGAGAT GGTATCTCGG AAGGAAACCA
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                        5210       5220       5230       5240       5250
refSn           CGCAGATGTC CACTGACCAG GAAGAAGTTA CTGGAATCGG TGATGATGCG
Thai-HP-1-Sn    CGCAGATGTC CACTGACCAG GAAGAAGTTA CTGGAATCGG TGATGATGCG
Thai-EU-1-Sn    CGCAGATGTC CACTGACCAG GAAGAAGTTA CTGGAATCGG TGATGATGCG
Thai-US-1-Sn    CGCAGATGTC CACTGACCAG GAAGAAGTTA CTGGAATCGG TGATGATGCG

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                        5260       5270       5280       5290       5300
refSn           GGCTCTGTGA ACCAGGCGGC ATTTGATCCT GCCCACCTCT GTGAAAACAC
Thai-HP-1-Sn    GGCTCTGTGA ACCAGGCGGC ATTTGATCCT GCCCACCTCT GTGAAAACAC
Thai-EU-1-Sn    GGCTCTGTGA ACCAGGCGGC ATTTGATCCT GCCCACCTCT GTGAAAACAC
Thai-US-1-Sn    GGCTCTGTGA ACCAGGCGGC ATTTGATCCT GCCCACCTCT GTGAAAACAC

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                       5310       5320
refSn           ACAGTCTGTG AAAAGCACAG TCTGA
Thai-HP-1-Sn    ACAGTCTGTG AAAAGCACAG TCTGA
Thai-EU-1-Sn    ACAGTCTGTG AAAAGCACAG TCTGA
Thai-US-1-Sn    ACAGTCTGTG AAAAGCACAG TCTGA
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Figure 4.9 Nucleotide sequence alignments of the reference Sn (refSn) (accession number is NM_214346) with recombinant plasmids of Thai-HP-1-pCR®-XL-TOPO®-Sn (Thai-HP-1-Sn), Thai-EU-1-pCR®-XL-TOPO®-Sn (Thai-EU-1-Sn) and Thai-US-1-pCR®-XL-TOPO®-Sn (Thai-US-1-Sn), respectively. The nucleotide changes are boxed.

Table 4.4 Deduced amino acid sequences of 3 recombinant porcine Sn (full) plasmids

Transformant's name	Sequence
1. Thai-HP-1-Sn (1,730 aa)	MDFLLLLLLLASSALAGLASWTVSSPETVQGIKGSCLIIPCTFRFPANVEVP HGITAIWYYDYSGKRLVVSHSRNPKVVENHFQGRALLLGQVEQRTCSLLL KDLQPQDSGSYNFRFEISEGNRWSDVKGTVVTVTEVPSVPTIALPAKLHE GMEVDFNCSTPYVCPTEPVNLQWQGQDPTRSVTSHLQKLEPSGTSHME TLHMALSWQDHGRILSCQVSAAERRMQKEIHLQVQYAPKGVEILFSHSG RNVLPGDLVTLSCQVNSSNPQVSSVQWVKDGTKLKDQKRVLQLRRAAW ADAGVYTCQAGNAVGSSVSPPVSLHVFMAEVQVSPVGSILENQTVTLAC NTPKEAPSELRYSWYKNHALLEGSHSRTLRLHSVTRADSGFYFCEVQNA RGRERSPPVSVVVSHPPLTPDLTAFLETQAGLVGILQCSVVSEPPATLVLS HGGLILASTSGEGDHSPRFSVASAPNSLRLEIQDLGPTDSGEYMCSASSS LGNASSTLDFHANAARLLISPAAEVVEGQAVTLSCRSSLSLMPDTRFSWY LNGALILEGPSSSLLLPAASSTDAGSYHCRAQNSHSTSGPSSPAVLTVLY APRQPVFTAQLDPDTAGAGAGRQGLLLCRVDSDPPAQLQLLHRGRVVA SSLSWGGGCCTCGGCFHRMKVTKAPNLLRVEIRDPVLEDEGVYLCEAS SALGNASASATLDAQATVLVITPSHTLQEGIEANLTCNVSREASGPANFS WFRDGALWAQGPLDTVTLLPVARTDAALYACRIVTEAGAGLSTPVALNV LYPPDPPKLSALLDVDQGHTAVFVCTVDSRPLAQLALFRGEHLLAASSAL RLPPRGRLQAKASANSLQLEVRDLSLGDSGSYHCEATNILGSANTSLTF QVRGAWVRVSPSPELQEGQAVVLSCQVPIGVLEGTSYRWYRDGQPLQE STSATLRFAAITLSQAGAYHCQAQAPGSATTDLAAPVSLHVTYAPRQATL TTLMDSGLGRLGLLLCRVNSDPPAQLRLLHGSRLVASTLQGVEELAGSS PRLQVATAPNTLRLEIHNAVLEDEGVYTCEATNTLGQTLASAAFDAQAMR VQVWPNATVQEGQLVNLTCLVWTTHLAQLTYTWYRDQQQLPGAAHSIL LPNVTVTDAASYRCGILIPGQALRLSRPVALDVLYAPRRLRLTHLLESRGG QLAVVLCTVDSRPAAQLTLSHAGRLLASSTAASVPNTLRLELWEPRPSDE

	GLYSCSARSPLGQANTSLELRLEGVQVALAPSATVPEGAPVTVTCEDPAA RPPTLYVWYHNSRWLQEGSAASLSFPAATRAHAGAYTCQVQDAQGTRI SQPAALHILYAPRDAVLSSFWDSRASPMAVVQCTVDSEPPAEMTLSHDG KVLATSHGVHGLAVGTGHVQVARNALQLRVQNVPSRDKDTYVCMDRNS SGSVSTMGQLQPEGVHVVAEPGLDVPEGTALNLSCRLPSGPGHIGNSTF AWFRNGRQLHTESVPTLTFTHVARAQAGLYHCQAELPAGAATSAPVLLR VLYPPKTPTMTVFVEPEGGIQGILDCRVDSEPLASLTLHLGSRLVASSQPQ AAPAKPHIRVSASPNALRVDMEELKPSDQGEYVCSASNALGSASAATYF GTRALHRLHLFQHLLWFLGLLASLLFLLLGLGVWYAWRRGNFYKLRMGE YSVEMVSRKETTQMSTDQEEVTGIGDDAGSVNQAAFDPAHLCENTQSV KSTV
2. Thai-EU-1-Sn (1,730 aa)	MDFLLLLLLLASSALAGLASWTVSSPETVQGIKGSCLIIPCTFGFPANVEVP HGITAIWYYDYSGKRLVVSHSRNPKVVENHFQGRALLLGQVEQRTCSLLL KDLQPQDSGSYNFRFEISEGNRWSDVKGTVVTVTEVPSVPTIALPAKLHE GMEVDFNCSTPYVCPTEPVNLQWQGQDPTRSVTSHLQKLEPSGTSHME TLHMALSWQDHGRILSCQVSAAERRMQKEIHLQVQYAPKGVEILFSHSG RNVLPGDLVTLSCQVNSSNPQVSSVQWVKDGTKLKDQKRVLQLRRAAW ADAGVYTCQAGNAVGSSVSPPVSLHVFMAEVQVSPVGSILENQTVTLAC NTPKEAPSELRYSWYKNHALLEGSHSRTLRLHSVTRADSGFYFCEVQNA RGRERSPPVSVVVSHPPLTPDLTAFLETQAGLVGILQCSVVSEPPATLVLS HGGLILASTSGEGDHSPRFSVASAPNSLRLEIQDLGPTDSGEYMCSASSS LGNASSTLDFHANAARLLISPAAEVVEGQAVTLSCRSSLSLMPDTRFSWY LNGALILEGPSSSLLLPAASSTDAGSYHCRAQNSHSTSGPSSPAVLTVLY APRQPVFTAQLDPDTAGAGAGRQGLLLCRVDSDPPAQLQLLHRGRVVA SSLSWGGGCCTCGGCFHRMKVTKAPNLLRVEIRDPVLEDEGVYLCEAS SALGNASASATLDAQATVLVITPSHTLQEGIEANLTCNVSREASGPANFS WFRDGALWAQGPLDTVTLLPVARTDAALYACRIVTEAGAGLSTPVALNV LYPPDPPKLSALLDVDQGHTAVFVCTVDSRPLAQLALFRGEHLLAASSAL

	RLPPRGRLQAKASANSLQLEVRDLSLGDSGSYHCEATNILGSANTSLTF QVRGAWVRVSPSPELQEGQAVVLSCQVPIGVLEGTSYRWYRDGQPLQE STSATLRFAAITLSQAGAYHCQAQAPGSATTDLAAPVSLHVTYAPRQATL TTLMDSGLGRLGLLLCRVNSDPPAQLRLLHGSRLVASTLQGVEELAGSS PRLQVATAPNTLRLEIHNAVLEDEGVYTCEATNTLGQTLASAAFDAQAMR VQVWPNATVQEGQLVNLTCLVWTTHLAQLTYTWYRDQQQLPGAAHSIL LPNVTVTDAASYRCGILIPGQALRLSRPVALDVLYAPRRLRLTHLLESRGG QLAVVLCTVDSRPAAQLTLSHAGRLLASSTAASVPNTLRLELWEPRPSDE GLYSCSARSPLGQANTSLELRLEGVQVALAPSATVPEGAPVTVTCEDPAA RPPTLYVWYHNSRWLQEGSAASLSFPAATRAHAGAYTCQVQDAQGTRI SQPAALHILYAPRDAVLSSFWDSRASPMAVVQCTVDSEPPAEMTLSHDG KVLATSHGVHGLAVGTGHVQVARNALQLRVQNVPSRDKDTYVCMDRNS LGSVSTMGQLQPEGVHVVAEPGLDVPEGTALNLSCRLPSGPGHIGNSTF AWFRNGRQLHTESVPTLTFTHVARAQAGLYHCQAELPAGAATSAPVLLR VLYPPKTPTMTVFVEPEGGIQGILDCRVDSEPLASLTLHLGSRLVASSQPQ AAPAKPHIRVSASPNALRVDMEELKPSDQGEYVCSASNALGSASAATYF GTRALHRLHLFQHLLWFLGLLASLLFLLLGLGVWYAWRRGNFYKLRMGE YSVEMVSRKETTQMSTDQEEVTGIGDDAGSVNQAAFDPAHLCENTQSV KSTV
3. Thai-US-1-Sn (1,730 aa)	MDFLLLLLLLASSALAGLASWTVSSPETVQGIKGSCLIIPCTFRFPANVEVP HGITAIWYYDYSGKRLVVSHSRNPKVVENHFQGRALLLGQVEQRTCSLLL KDLQPQDSGSYNFRFEISEGNRWSDVKGTVVTVTEVPSVPTIALPAKLHE GMEVDFNCSTPYVCPTEPVNLQWQGQDPTRSVTSHLQKLEPSGTSHME TLHMALSWQDHGRILSCQVSAAERRMQKEIHLQVQYAPKGVEILFSHSG RNVLPGDLVTLSCQVNSSNPQVSSVQWVKDGTKLKDQKRVLQLRRAAW ADAGVYTCQAGNAVGSSVSPPVSLHVFMAEVQVSPVGSILENQTVTLAC NTPKEAPSELRYSWYKNHALLEGSHSRTLRLHSVTRADSGFYFCEVQNA RGRERSPPVSVVVSHPPLTPDLTAFLETQAGLVGILQCSVVSEPPATLVLS

HGGLILASTSGEGDHSPRFSVASAPNSLRLEIQDLGPTDSGEYMCSASSS
LGNASSTLDFHANAARLLISPAAEVVEGQAVTLSCRSSLSLMPDTRFSWY
LNGALILEGPSSSLLLPAASSTDAGSYHCRAQNSHSTSGPSSPAVLTVLY
APRQPVFTAQLDPDTAGAGAGRQGLLLCRVDSDPPAQLQLLHRGRVVA
SSLSWGGGCCTCGGCFHRMKVTKAPNLLRVEIRDPVLEDEGVYLCEAS
SALGNASASATLDAQATVLVITPSHTLQEGIEANLTCNVSREASGPANFS
WFRDGALWAQGPLDTVTLLPVARTDAALYACRIVTEAGAGLSTPVALNV
LYPPDPPKLSALLDVDQGHTAVFVCTVDSRPLAQLALFRGEHLLAASSAL
RLPPRGRLQAKASANSLQLEVRDLSLGDSGSYHCEATNILGSANTSLTF
QVRGAWVRVSPSPELQEGQAVVLSCQVPIGVLEGTSYRWYRDGQPLQE
STSATLRFAAITLSQAGAYHCQAQAPGSATTDLAAPVSLHVTYAPRQATL
TTLMDSGLGRLGLLLCRVNSDPPAQLRLLHGSRLVASTLQGVEELAGSS
PRLQVATAPNTLRLEIHNAVLEDEGVYTCEATNTLGQTLASAAFDAQAMR
VQVWPNATVQEGQLVNLTCLVWTTHLAQLTYTWYRDQQQLPGAAHSIL
LPNVTVTDAASYRCGILIPGQALRLSRPVALDVLYAPRRLRLTHLLESRGG
QLAVVLCTVDSRPAAQLTLSHAGRLLASSTAASVPNTLRLELWEPRPSDE
GLYSCSARSPLGQANTSLELRLEGVQVALAPSATVPEGAPVTVTCEDPAA
RPPTLYVWYHNSRWLQEGSAASLSFPAATRAHAGAYTCQVQDAQGTRI
SQPAALHILYAPRDAVLSSFWDSRASPMAVVQCTVDSEPPAEMTLSHDG
KVLATSHGVHGLAVGTGHVQVARNALQLRVQNVPSRDKDTYVCMDRNS
SGSVSTMGQLQPEGVHVVAEPGLDVPEGTALNLSCRLPSGPGHIGNSTF
AWFRNGRQLHTESVPTLTFTHVARAQAGLYHCQAELPAGAATSAPVLLR
VLYPPKTPTMTVFVEPEGGIQGILDCRVDSEPLASLTLHLGSRLVASSQPQ
AAPAKPHIRVSASPNALRVDMEELKPSDQGEYVCSASNALGSASAATYF
GTRALHRLHLFQHLLWFLGLLASLLFLLLGLGVWYAWRRGNFYKLRMGE
YSVEMVSRKETTQMSTDQEEVTGIGDDAGSVNQAAFDPAHLCENTQSV
KSTV

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                         10         20         30         40         50
refSn           MDFLLLLLLL ASSALAGLAS WTVSSPETVQ GIKGSCLIIP CTFGFPANVE
Thai-HP-1-Sn    MDFLLLLLLL ASSALAGLAS WTVSSPETVQ GIKGSCLIIP CTFRFPANVE
Thai-EU-1-Sn    MDFLLLLLLL ASSALAGLAS WTVSSPETVQ GIKGSCLIIP CTFGFPANVE
Thai-US-1-Sn    MDFLLLLLLL ASSALAGLAS WTVSSPETVQ GIKGSCLIIP CTFRFPANVE

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                          60         70         80         90        100
refSn           VPHGITAIWY YDYSGKRLVV SHSRNPKVVE NHFQGRALLL GQVEQRTCSL
Thai-HP-1-Sn    VPHGITAIWY YDYSGKRLVV SHSRNPKVVE NHFQGRALLL GQVEQRTCSL
Thai-EU-1-Sn    VPHGITAIWY YDYSGKRLVV SHSRNPKVVE NHFQGRALLL GQVEQRTCSL
Thai-US-1-Sn    VPHGITAIWY YDYSGKRLVV SHSRNPKVVE NHFQGRALLL GQVEQRTCSL

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                         110        120        130        140        150
refSn           LLKDLQPQDS GSYNFRFEIS EGNRWSDVKG TVVTVTEVPS VPTIALPAKL
Thai-HP-1-Sn    LLKDLQPQDS GSYNFRFEIS EGNRWSDVKG TVVTVTEVPS VPTIALPAKL
Thai-EU-1-Sn    LLKDLQPQDS GSYNFRFEIS EGNRWSDVKG TVVTVTEVPS VPTIALPAKL
Thai-US-1-Sn    LLKDLQPQDS GSYNFRFEIS EGNRWSDVKG TVVTVTEVPS VPTIALPAKL

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                         160        170        180        190        200
refSn           HEGMEVDFNC STPYVCPTEP VNLQWQGQDP TRSVTSHLQK LEPSGTSHME
Thai-HP-1-Sn    HEGMEVDFNC STPYVCPTEP VNLQWQGQDP TRSVTSHLQK LEPSGTSHME
Thai-EU-1-Sn    HEGMEVDFNC STPYVCPTEP VNLQWQGQDP TRSVTSHLQK LEPSGTSHME
Thai-US-1-Sn    HEGMEVDFNC STPYVCPTEP VNLQWQGQDP TRSVTSHLQK LEPSGTSHME

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                         210        220        230        240        250
refSn           TLHMALSWQD HGRILSCQVS AAERRMQKEI HLQVQYAPKG VEILFSHSGR
Thai-HP-1-Sn    TLHMALSWQD HGRILSCQVS AAERRMQKEI HLQVQYAPKG VEILFSHSGR
Thai-EU-1-Sn    TLHMALSWQD HGRILSCQVS AAERRMQKEI HLQVQYAPKG VEILFSHSGR
Thai-US-1-Sn    TLHMALSWQD HGRILSCQVS AAERRMQKEI HLQVQYAPKG VEILFSHSGR

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                         260        270        280        290        300
refSn           NVLPGDLVTL SCQVNSSNPQ VSSVQWVKDG TKLKDQKRVL QLRRAAWADA
Thai-HP-1-Sn    NVLPGDLVTL SCQVNSSNPQ VSSVQWVKDG TKLKDQKRVL QLRRAAWADA
Thai-EU-1-Sn    NVLPGDLVTL SCQVNSSNPQ VSSVQWVKDG TKLKDQKRVL QLRRAAWADA
Thai-US-1-Sn    NVLPGDLVTL SCQVNSSNPQ VSSVQWVKDG TKLKDQKRVL QLRRAAWADA

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                         310        320        330        340        350
refSn           GVYTCQAGNA VGSSVSPPVS LHVFMAEVQV SPVGSILENQ TVTLACNTPK
Thai-HP-1-Sn    GVYTCQAGNA VGSSVSPPVS LHVFMAEVQV SPVGSILENQ TVTLACNTPK
Thai-EU-1-Sn    GVYTCQAGNA VGSSVSPPVS LHVFMAEVQV SPVGSILENQ TVTLACNTPK
Thai-US-1-Sn    GVYTCQAGNA VGSSVSPPVS LHVFMAEVQV SPVGSILENQ TVTLACNTPK

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                         360        370        380        390        400
refSn           EAPSELRYSW YKNHALLEGS HSRTLRLHSV TRADSGFYFC EVQNARGRER
Thai-HP-1-Sn    EAPSELRYSW YKNHALLEGS HSRTLRLHSV TRADSGFYFC EVQNARGRER
Thai-EU-1-Sn    EAPSELRYSW YKNHALLEGS HSRTLRLHSV TRADSGFYFC EVQNARGRER
Thai-US-1-Sn    EAPSELRYSW YKNHALLEGS HSRTLRLHSV TRADSGFYFC EVQNARGRER
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                         410        420        430        440        450
refSn           SPPVSVVVSH PPLTPDLTAF LETQAGLVGI LQCSVVSEPP ATLVLSHGGL
Thai-HP-1-Sn    SPPVSVVVSH PPLTPDLTAF LETQAGLVGI LQCSVVSEPP ATLVLSHGGL
Thai-EU-1-Sn    SPPVSVVVSH PPLTPDLTAF LETQAGLVGI LQCSVVSEPP ATLVLSHGGL
Thai-US-1-Sn    SPPVSVVVSH PPLTPDLTAF LETQAGLVGI LQCSVVSEPP ATLVLSHGGL

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                         460        470        480        490        500
refSn           ILASTSGEGD HSPRFSVASA PNSLRLEIQD LGPTDSGEYM CSASSSLGNA
Thai-HP-1-Sn    ILASTSGEGD HSPRFSVASA PNSLRLEIQD LGPTDSGEYM CSASSSLGNA
Thai-EU-1-Sn    ILASTSGEGD HSPRFSVASA PNSLRLEIQD LGPTDSGEYM CSASSSLGNA
Thai-US-1-Sn    ILASTSGEGD HSPRFSVASA PNSLRLEIQD LGPTDSGEYM CSASSSLGNA

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                         510        520        530        540        550
refSn           SSTLDFHANA ARLLISPAAE VVEGQAVTLS CRSSLSLMPD TRFSWYLNGA
Thai-HP-1-Sn    SSTLDFHANA ARLLISPAAE VVEGQAVTLS CRSSLSLMPD TRFSWYLNGA
Thai-EU-1-Sn    SSTLDFHANA ARLLISPAAE VVEGQAVTLS CRSSLSLMPD TRFSWYLNGA
Thai-US-1-Sn    SSTLDFHANA ARLLISPAAE VVEGQAVTLS CRSSLSLMPD TRFSWYLNGA

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                         560        570        580        590        600
refSn           LILEGPSSSL LLPAASSTDA GSYHCRAQNS HSTSGPSSPA VLTVLYAPRQ
Thai-HP-1-Sn    LILEGPSSSL LLPAASSTDA GSYHCRAQNS HSTSGPSSPA VLTVLYAPRQ
Thai-EU-1-Sn    LILEGPSSSL LLPAASSTDA GSYHCRAQNS HSTSGPSSPA VLTVLYAPRQ
Thai-US-1-Sn    LILEGPSSSL LLPAASSTDA GSYHCRAQNS HSTSGPSSPA VLTVLYAPRQ

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                         610        620        630        640        650
refSn           PVFTAQLDPD TAGAGAGRQG LLLCRVDSDP PAQLQLLHRG RVVASSLSWG
Thai-HP-1-Sn    PVFTAQLDPD TAGAGAGRQG LLLCRVDSDP PAQLQLLHRG RVVASSLSWG
Thai-EU-1-Sn    PVFTAQLDPD TAGAGAGRQG LLLCRVDSDP PAQLQLLHRG RVVASSLSWG
Thai-US-1-Sn    PVFTAQLDPD TAGAGAGRQG LLLCRVDSDP PAQLQLLHRG RVVASSLSWG

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                         660        670        680        690        700
refSn           GGCCTCGGCF HRMKVTKAPN LLRVEIRDPV LEDEGVYLCE ASSALGNASA
Thai-HP-1-Sn    GGCCTCGGCF HRMKVTKAPN LLRVEIRDPV LEDEGVYLCE ASSALGNASA
Thai-EU-1-Sn    GGCCTCGGCF HRMKVTKAPN LLRVEIRDPV LEDEGVYLCE ASSALGNASA
Thai-US-1-Sn    GGCCTCGGCF HRMKVTKAPN LLRVEIRDPV LEDEGVYLCE ASSALGNASA

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                         710        720        730        740        750
refSn           SATLDAQATV LVITPSHTLQ EGIEANLTCN VSREASGPAN FSWFRDGALW
Thai-HP-1-Sn    SATLDAQATV LVITPSHTLQ EGIEANLTCN VSREASGPAN FSWFRDGALW
Thai-EU-1-Sn    SATLDAQATV LVITPSHTLQ EGIEANLTCN VSREASGPAN FSWFRDGALW
Thai-US-1-Sn    SATLDAQATV LVITPSHTLQ EGIEANLTCN VSREASGPAN FSWFRDGALW

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                         760        770        780        790        800
refSn           AQGPLDTVTL LPVARTDAAL YACRIVTEAG AGLSTPVALN VLYPPDPPKL
Thai-HP-1-Sn    AQGPLDTVTL LPVARTDAAL YACRIVTEAG AGLSTPVALN VLYPPDPPKL
Thai-EU-1-Sn    AQGPLDTVTL LPVARTDAAL YACRIVTEAG AGLSTPVALN VLYPPDPPKL
Thai-US-1-Sn    AQGPLDTVTL LPVARTDAAL YACRIVTEAG AGLSTPVALN VLYPPDPPKL
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                        810        820        830        840        850
refSn           SALLDVDQGH TAVFVCTVDS RPLAQLALFR GEHLLAASSA LRLPPRGRLQ
Thai-HP-1-Sn    SALLDVDQGH TAVFVCTVDS RPLAQLALFR GEHLLAASSA LRLPPRGRLQ
Thai-EU-1-Sn    SALLDVDQGH TAVFVCTVDS RPLAQLALFR GEHLLAASSA LRLPPRGRLQ
Thai-US-1-Sn    SALLDVDQGH TAVFVCTVDS RPLAQLALFR GEHLLAASSA LRLPPRGRLQ

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                        860        870        880        890        900
refSn           AKASANSLQL EVRDLSLGDS GSYHCEATNI LGSANTSLTF QVRGAWVRVS
Thai-HP-1-Sn    AKASANSLQL EVRDLSLGDS GSYHCEATNI LGSANTSLTF QVRGAWVRVS
Thai-EU-1-Sn    AKASANSLQL EVRDLSLGDS GSYHCEATNI LGSANTSLTF QVRGAWVRVS
Thai-US-1-Sn    AKASANSLQL EVRDLSLGDS GSYHCEATNI LGSANTSLTF QVRGAWVRVS

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                        910        920        930        940        950
refSn           PSPELQEGQA VVLSCQVPIG VLEGTSYRWY RDGQPLQEST SATLRFAAIT
Thai-HP-1-Sn    PSPELQEGQA VVLSCQVPIG VLEGTSYRWY RDGQPLQEST SATLRFAAIT
Thai-EU-1-Sn    PSPELQEGQA VVLSCQVPIG VLEGTSYRWY RDGQPLQEST SATLRFAAIT
Thai-US-1-Sn    PSPELQEGQA VVLSCQVPIG VLEGTSYRWY RDGQPLQEST SATLRFAAIT

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                       960        970        980        990       1000
refSn           LSQAGAYHCQ AQAPGSATTD LAAPVSLHVT YAPRQATLTT LMDSGLGRLG
Thai-HP-1-Sn    LSQAGAYHCQ AQAPGSATTD LAAPVSLHVT YAPRQATLTT LMDSGLGRLG
Thai-EU-1-Sn    LSQAGAYHCQ AQAPGSATTD LAAPVSLHVT YAPRQATLTT LMDSGLGRLG
Thai-US-1-Sn    LSQAGAYHCQ AQAPGSATTD LAAPVSLHVT YAPRQATLTT LMDSGLGRLG

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                       1010       1020       1030       1040       1050
refSn           LLLCRVNSDP PAQLRLLHGS RLVASTLQGV EELAGSSPRL QVATAPNTLR
Thai-HP-1-Sn    LLLCRVNSDP PAQLRLLHGS RLVASTLQGV EELAGSSPRL QVATAPNTLR
Thai-EU-1-Sn    LLLCRVNSDP PAQLRLLHGS RLVASTLQGV EELAGSSPRL QVATAPNTLR
Thai-US-1-Sn    LLLCRVNSDP PAQLRLLHGS RLVASTLQGV EELAGSSPRL QVATAPNTLR

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                       1060       1070       1080       1090       1100
refSn           LEIHNAVLED EGVYTCEATN TLGQTLASAA FDAQAMRVQV WPNATVQEGQ
Thai-HP-1-Sn    LEIHNAVLED EGVYTCEATN TLGQTLASAA FDAQAMRVQV WPNATVQEGQ
Thai-EU-1-Sn    LEIHNAVLED EGVYTCEATN TLGQTLASAA FDAQAMRVQV WPNATVQEGQ
Thai-US-1-Sn    LEIHNAVLED EGVYTCEATN TLGQTLASAA FDAQAMRVQV WPNATVQEGQ

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                       1110       1120       1130       1140       1150
refSn           LVNLTCLVWT THLAQLTYTW YRDQQQLPGA AHSILLPNVT VTDAASYRCG
Thai-HP-1-Sn    LVNLTCLVWT THLAQLTYTW YRDQQQLPGA AHSILLPNVT VTDAASYRCG
Thai-EU-1-Sn    LVNLTCLVWT THLAQLTYTW YRDQQQLPGA AHSILLPNVT VTDAASYRCG
Thai-US-1-Sn    LVNLTCLVWT THLAQLTYTW YRDQQQLPGA AHSILLPNVT VTDAASYRCG


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                       1160       1170       1180       1190       1200
refSn           ILIPGQALRL SRPVALDVLY APRRLRLTHL LESRGGQLAV VLCTVDSRPA
Thai-HP-1-Sn    ILIPGQALRL SRPVALDVLY APRRLRLTHL LESRGGQLAV VLCTVDSRPA
Thai-EU-1-Sn    ILIPGQALRL SRPVALDVLY APRRLRLTHL LESRGGQLAV VLCTVDSRPA
Thai-US-1-Sn    ILIPGQALRL SRPVALDVLY APRRLRLTHL LESRGGQLAV VLCTVDSRPA
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                        1210       1220       1230       1240       1250
refSn           AQLTLSHAGR LLASSTAASV PNTLRLELWE PRPSDEGLYS CSARSPLGQA
Thai-HP-1-Sn    AQLTLSHAGR LLASSTAASV PNTLRLELWE PRPSDEGLYS CSARSPLGQA
Thai-EU-1-Sn    AQLTLSHAGR LLASSTAASV PNTLRLELWE PRPSDEGLYS CSARSPLGQA
Thai-US-1-Sn    AQLTLSHAGR LLASSTAASV PNTLRLELWE PRPSDEGLYS CSARSPLGQA

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                        1260       1270       1280       1290       1300
refSn           NTSLELRLEG VQVALAPSAT VPEGAPVTVT CEDPAARPPT LYVWYHNSRW
Thai-HP-1-Sn    NTSLELRLEG VQVALAPSAT VPEGAPVTVT CEDPAARPPT LYVWYHNSRW
Thai-EU-1-Sn    NTSLELRLEG VQVALAPSAT VPEGAPVTVT CEDPAARPPT LYVWYHNSRW
Thai-US-1-Sn    NTSLELRLEG VQVALAPSAT VPEGAPVTVT CEDPAARPPT LYVWYHNSRW

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                        1310       1320       1330       1340       1350
refSn           LQEGSAASLS FPAATRAHAG AYTCQVQDAQ GTRISQPAAL HILYAPRDAV
Thai-HP-1-Sn    LQEGSAASLS FPAATRAHAG AYTCQVQDAQ GTRISQPAAL HILYAPRDAV
Thai-EU-1-Sn    LQEGSAASLS FPAATRAHAG AYTCQVQDAQ GTRISQPAAL HILYAPRDAV
Thai-US-1-Sn    LQEGSAASLS FPAATRAHAG AYTCQVQDAQ GTRISQPAAL HILYAPRDAV

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        1360       1370       1380       1390       1400
refSn           LSSFWDSRAS PMAVVQCTVD SEPPAEMTLS HDGKVLATSH GVHGLAVGTG
Thai-HP-1-Sn    LSSFWDSRAS PMAVVQCTVD SEPPAEMTLS HDGKVLATSH GVHGLAVGTG
Thai-EU-1-Sn    LSSFWDSRAS PMAVVQCTVD SEPPAEMTLS HDGKVLATSH GVHGLAVGTG
Thai-US-1-Sn    LSSFWDSRAS PMAVVQCTVD SEPPAEMTLS HDGKVLATSH GVHGLAVGTG

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                        1410       1420       1430       1440       1450
refSn           HVQVARNALQ LRVQNVPSRD KDTYVCMDRN SLGSVSTMGQ LQPEGVHVVA
Thai-HP-1-Sn    HVQVARNALQ LRVQNVPSRD KDTYVCMDRN SSGSVSTMGQ LQPEGVHVVA
Thai-EU-1-Sn    HVQVARNALQ LRVQNVPSRD KDTYVCMDRN SLGSVSTMGQ LQPEGVHVVA
Thai-US-1-Sn    HVQVARNALQ LRVQNVPSRD KDTYVCMDRN SSGSVSTMGQ LQPEGVHVVA

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        1460       1470       1480       1490       1500
refSn           EPGLDVPEGT ALNLSCRLPS GPGHIGNSTF AWFRNGRQLH TESVPTLTFT
Thai-HP-1-Sn    EPGLDVPEGT ALNLSCRLPS GPGHIGNSTF AWFRNGRQLH TESVPTLTFT
Thai-EU-1-Sn    EPGLDVPEGT ALNLSCRLPS GPGHIGNSTF AWFRNGRQLH TESVPTLTFT
Thai-US-1-Sn    EPGLDVPEGT ALNLSCRLPS GPGHIGNSTF AWFRNGRQLH TESVPTLTFT

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        1510       1520       1530       1540       1550
refSn           HVARAQAGLY HCQAELPAGA ATSAPVLLRV LYPPKTPTMT VFVEPEGGIQ
Thai-HP-1-Sn    HVARAQAGLY HCQAELPAGA ATSAPVLLRV LYPPKTPTMT VFVEPEGGIQ
Thai-EU-1-Sn    HVARAQAGLY HCQAELPAGA ATSAPVLLRV LYPPKTPTMT VFVEPEGGIQ
Thai-US-1-Sn    HVARAQAGLY HCQAELPAGA ATSAPVLLRV LYPPKTPTMT VFVEPEGGIQ

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        1560       1570       1580       1590       1600
refSn           GILDCRVDSE PLASLTLHLG SRLVASSQPQ AAPAKPHIRV SASPNALRVD
Thai-HP-1-Sn    GILDCRVDSE PLASLTLHLG SRLVASSQPQ AAPAKPHIRV SASPNALRVD
Thai-EU-1-Sn    GILDCRVDSE PLASLTLHLG SRLVASSQPQ AAPAKPHIRV SASPNALRVD
Thai-US-1-Sn    GILDCRVDSE PLASLTLHLG SRLVASSQPQ AAPAKPHIRV SASPNALRVD
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                ....|....| ....|....| ....|....| ....|....| ....|....|
                       1610       1620       1630       1640       1650
refSn           MEELKPSDQG EYVCSASNAL GSASAATYFG TRALHRLHLF QHLLWFLGLL
Thai-HP-1-Sn    MEELKPSDQG EYVCSASNAL GSASAATYFG TRALHRLHLF QHLLWFLGLL
Thai-EU-1-Sn    MEELKPSDQG EYVCSASNAL GSASAATYFG TRALHRLHLF QHLLWFLGLL
Thai-US-1-Sn    MEELKPSDQG EYVCSASNAL GSASAATYFG TRALHRLHLF QHLLWFLGLL

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       1660       1670       1680       1690       1700
refSn           ASLLFLLLGL GVWYAWRRGN FYKLRMGEYS VEMVSRKETT QMSTDQEEVT
Thai-HP-1-Sn    ASLLFLLLGL GVWYAWRRGN FYKLRMGEYS VEMVSRKETT QMSTDQEEVT
Thai-EU-1-Sn    ASLLFLLLGL GVWYAWRRGN FYKLRMGEYS VEMVSRKETT QMSTDQEEVT
Thai-US-1-Sn    ASLLFLLLGL GVWYAWRRGN FYKLRMGEYS VEMVSRKETT QMSTDQEEVT

                 ....|....| ....|....| ....|....|
                        1710       1720       1730
refSn            GIGDDAGSVN QAAFDPAHLC ENTQSVKSTV
Thai-HP-1-Sn     GIGDDAGSVN QAAFDPAHLC ENTQSVKSTV
Thai-EU-1-Sn     GIGDDAGSVN QAAFDPAHLC ENTQSVKSTV
Thai-US-1-Sn     GIGDDAGSVN QAAFDPAHLC ENTQSVKSTV
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Figure 4.10 Deduced amino acid alignments of the reference Sn (refSn) (accession number is NP_999511) with recombinant plasmids of Thai-HP-1-pCR®-XL-TOPO®-Sn (Thai-HP-1-Sn), Thai-EU-1-pCR®-XL-TOPO®-Sn (Thai-EU-1-Sn) and Thai-US-1-pCR®-XL-TOPO®-Sn (Thai-US-1-Sn), respectively. The amino acid changes are boxed.

2.2 Nucleotide and deduced amino acid sequences of recombinant porcine Sn (N-terminal domain) plasmids

The author selected 3 recombinant plasmids, Thai-HP-1-pCR®8-GW-TOPO®-N-terminal Sn (Thai-HP-1-N-terminal-Sn), Thai-EU-1-pCR®8-GW-TOPO®-N-terminal Sn (Thai-EU-1-N-terminal-Sn) and Thai-US-1-pCR®8-GW-TOPO®-N-terminal Sn (Thai-US-1-N-terminal-Sn), respectively, to sequence. The complete sequence of 3 recombinant porcine Sn (N-terminal domain) plasmids were successfully sequenced at the same length of 1,024 bp (Table 4.5) and were analyzed the alignment with the available data bases of refSn cDNA (accession number is NM_214346) form the PAM of normal pigs as shown in Figure 4.11. The results showed that the nucleotide sequences of 3 recombinant porcine Sn (N-terminal domain) plasmids were changed in 2 positions of 130 and 762.

The deduced amino acid sequences and alignment of recombinant porcine Sn (N-terminal domain) plasmids from PRRVS infected PAM (341 amino acids) and refSn cDNA (accession number is NP_999511) from normal PAM (341 amino acids) were shown in Table 4.6 and Figure 4.12, respectively. The results indicated that the amino acid sequences of 3 recombinant porcine Sn (N-terminal domain) plasmids were changed in a position of 44 (Glycine to Arginine).

Table 4.5 Nucleotide sequences of 3 recombinant porcine Sn (N-terminal domain) plasmids

Product's name	Sequence
1. Thai-HP-1-N-terminal-Sn (1,024 bp)	atggacttcctgctcctgctcctcctcctggcttcatctgctctagcaggcctggcctcgtggacggttt ccagccccgagaccgtgcagggcatcaagggctcctgcctcatcatcccctgcaccttccgcttcc cggccaacgtggaggtgccccatggcatcacagccatctggtactatgactactcaggcaagcgc ctggtagtgagccactccaggaacccaaaggtggtggagaaccacttccaaggccgggccctg ctgttggggcaggttgaacagaggacgtgcagcctgctgctgaaggacctgcagccccaggact cgggctcctataacttccgctttgagatcagcgagggcaaccgctggtcagatgtcaaaggcacag ttgtcaccgtgacagaggtgcccagcgtgcccaccattgccttgccagccaagctgcatgagggc atggaggtggacttcaactgctccactccctatgtgtgcccgacggagccggtcaacctacagtgg caaggccaggatcccacccgctccgtcacctcccacctccagaagcttgagccctcgggcacca gccacatggagaccctgcacatggccctgtcctggcaggaccatggccggatcctgagctgcca ggtctcagcagccgaacgcaggatgcagaaggagattcacctccaagtgcagtatgcccccaag ggtgtggagatccttttcagccactccggacggaacgtccttcctggtgatctggtcaccctcagctg ccaggtgaatagcagcaaccctcaggtcagttccgtgcagtgggtcaaggatgggacgaagctc aaagaccagaaacgtgtactgcagttgcgccgggcagcctgggctgatgctggcgtctacacctg ccaagccgggaatgccgtgggctcttcagtctcacccccggtcagcctccacgtcttcatggctga ggtccaggtaagccctgtgggctccatcctggagaaccagacgg
2. Thai-EU-1-N-terminal-Sn (1,024 bp)	atggacttcctgctcctgctcctcctcctggcttcatctgctctagcaggcctggcctcgtggacggttt ccagccccgagaccgtgcagggcatcaagggctcctgcctcatcatcccctgcaccttcggcttc ccggccaacgtggaggtgccccatggcatcacagccatctggtactatgactactcaggcaagcg cctggtagtgagccactccaggaacccaaaggtggtggagaaccacttccaaggccgggccct gctgttggggcaggttgaacagaggacgtgcagcctgctgctgaaggacctgcagccccaggac tcgggctcctataacttccgctttgagatcagcgagggcaaccgctggtcagatgtcaaaggcaca gttgtcaccgtgacagaggtgcccagcgtgcccaccattgccttgccagccaagctgcatgaggg catggaggtggacttcaactgctccactccctatgtgtgcccgacggagccggtcaacctacagtg

	gcaaggccaggatcccacccgctccgtcacctcccacctccagaagcttgagccctcgggcacc agccacatggagaccctgcacatggccctgtcctggcaggaccatggccggatcctgagctgcc aggtctcagcagccgaacgcaggatgcagaaggagattcacctccaagtgcagtatgcccccaa gggtgtggagatccttttcagccactccggacggaacgtccttccaggtgatctggtcaccctcagct gccaggtgaatagcagcaaccctcaggtcagttccgtgcagtgggtcaaggatgggacgaagct caaagaccagaaacgtgtactgcagttgcgccgggcagcctgggctgatgctggcgtctacacct gccaagccgggaatgccgtgggctcttcagtctcacccccggtcagcctccacgtcttcatggctg aggtccaggtaagccctgtgggctccatcctggagaaccagacgg
3. Thai-US-1- N-terminal-Sn (1,024 bp)	atggacttcctgctcctgctcctcctcctggcttcatctgctctagcaggcctggcctcgtggacggttt ccagccccgagaccgtgcagggcatcaagggctcctgcctcatcatcccctgcaccttccgcttcc cggccaacgtggaggtgccccatggcatcacagccatctggtactatgactactcaggcaagcgc ctggtagtgagccactccaggaacccaaaggtggtggagaaccacttccaaggccgggccctg ctgttggggcaggttgaacagaggacgtgcagcctgctgctgaaggacctgcagccccaggact cgggctcctataacttccgctttgagatcagcgagggcaaccgctggtcagatgtcaaaggcacag ttgtcaccgtgacagaggtgcccagcgtgcccaccattgccttgccagccaagctgcatgagggc atggaggtggacttcaactgctccactccctatgtgtgcccgacggagccggtcaacctacagtgg caaggccaggatcccacccgctccgtcacctcccacctccagaagcttgagccctcgggcacca gccacatggagaccctgcacatggccctgtcctggcaggaccatggccggatcctgagctgcca ggtctcagcagccgaacgcaggatgcagaaggagattcacctccaagtgcagtatgcccccaag ggtgtggagatccttttcagccactccggacggaacgtccttcctggtgatctggtcaccctcagctg ccaggtgaatagcagcaaccctcaggtcagttccgtgcagtgggtcaaggatgggacgaagctc aaagaccagaaacgtgtactgcagttgcgccgggcagcctgggctgatgctggcgtctacacctg ccaagccgggaatgccgtgggctcttcagtctcacccccggtcagcctccacgtcttcatggctga ggtccaggtaagccctgtgggctccatcctggagaaccagacgg

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                          ....|....| ....|....| ....|....| ....|....| ....|....|
                                  10         20         30         40         50
                                  M13 forward
refSn                     ---------- ---------- ---------- ---------- ----------
Thai-HP-1-N-terminal-Sn   GTAAAACGAC GGCCAGTCTT AAGCTCGGGC CCCAAATAAT GATTTTATTT
Thai-EU-1-N-terminal-Sn   GTAAAACGAC GGCCAGTCTT AAGCTCGGGC CCCAAATAAT GATTTTATTT
Thai-US-1-N-terminal-Sn   GTAAAACGAC GGCCAGTCTT AAGCTCGGGC CCCAAATAAT GATTTTATTT

                          ....|....| ....|....| ....|....| ....|....| ....|....|
                                  60         70         80         90        100
refSn                     ---------- ---------- ---------- ---------- ----------
Thai-HP-1-N-terminal-Sn   TGACTGATAG TGACCTGTTC GTTGCAACAA ATTGATGAGC AATGCTTTTT
Thai-EU-1-N-terminal-Sn   TGACTGATAG TGACCTGTTC GTTGCAACAA ATTGATGAGC AATGCTTTTT
Thai-US-1-N-terminal-Sn   TGACTGATAG TGACCTGTTC GTTGCAACAA ATTGATGAGC AATGCTTTTT

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                                 110        120        130        140        150
refSn                     ---------- ---------- ---------- ---------- ------ATGG
Thai-HP-1-N-terminal-Sn   TATAATGCCA ACTTTGTACA AAAAAGCAGG CTCCGAATTC GCCCTTATGG
Thai-EU-1-N-terminal-Sn   TATAATGCCA ACTTTGTACA AAAAAGCAGG CTCCGAATTC GCCCTTATGG
Thai-US-1-N-terminal-Sn   TATAATGCCA ACTTTGTACA AAAAAGCAGG CTCCGAATTC GCCCTTATGG

                          ....|....| ....|....| ....|....| ....|....| ....|....|
                                 160        170        180        190        200
refSn                     ACTTCCTGCT CCTGCTCCTC CTCCTGGCTT CATCTGCTCT AGCAGGCCTG
Thai-HP-1-N-terminal-Sn   ACTTCCTGCT CCTGCTCCTC CTCCTGGCTT CATCTGCTCT AGCAGGCCTG
Thai-EU-1-N-terminal-Sn   ACTTCCTGCT CCTGCTCCTC CTCCTGGCTT CATCTGCTCT AGCAGGCCTG
Thai-US-1-N-terminal-Sn   ACTTCCTGCT CCTGCTCCTC CTCCTGGCTT CATCTGCTCT AGCAGGCCTG

                          ....|....| ....|....| ....|....| ....|....| ....|....|
                                 210        220        230        240        250
refSn                     GCCTCGTGGA CGGTTTCCAG CCCCGAGACC GTGCAGGGCA TCAAGGGCTC
Thai-HP-1-N-terminal-Sn   GCCTCGTGGA CGGTTTCCAG CCCCGAGACC GTGCAGGGCA TCAAGGGCTC
Thai-EU-1-N-terminal-Sn   GCCTCGTGGA CGGTTTCCAG CCCCGAGACC GTGCAGGGCA TCAAGGGCTC
Thai-US-1-N-terminal-Sn   GCCTCGTGGA CGGTTTCCAG CCCCGAGACC GTGCAGGGCA TCAAGGGCTC

                          ....|....| ....|....| ....|....| ....|....| ....|....|
                                 260        270        280        290        300
refSn                     CTGCCTCATC ATCCCCTGCA CCTTCGGCTT CCCGGCCAAC GTGGAGGTGC
Thai-HP-1-N-terminal-Sn   CTGCCTCATC ATCCCCTGCA CCTTCGGCTT CCCGGCCAAC GTGGAGGTGC
Thai-EU-1-N-terminal-Sn   CTGCCTCATC ATCCCCTGCA CCTTCGGCTT CCCGGCCAAC GTGGAGGTGC
Thai-US-1-N-terminal-Sn   CTGCCTCATC ATCCCCTGCA CCTTCGGCTT CCCGGCCAAC GTGGAGGTGC

                          ....|....| ....|....| ....|....| ....|....| ....|....|
                                 310        320        330        340        350
refSn                     CCCATGGCAT CACAGCCATC TGGTACTATG ACTACTCAGG CAAGCGCCTG
Thai-HP-1-N-terminal-Sn   CCCATGGCAT CACAGCCATC TGGTACTATG ACTACTCAGG CAAGCGCCTG
Thai-EU-1-N-terminal-Sn   CCCATGGCAT CACAGCCATC TGGTACTATG ACTACTCAGG CAAGCGCCTG
Thai-US-1-N-terminal-Sn   CCCATGGCAT CACAGCCATC TGGTACTATG ACTACTCAGG CAAGCGCCTG

                          ....|....| ....|....| ....|....| ....|....| ....|....|
                                 360        370        380        390        400
refSn                     GTAGTGAGCC ACTCCAGGAA CCCAAAGGTG GTGGAGAACC ACTTCCAAGG
Thai-HP-1-N-terminal-Sn   GTAGTGAGCC ACTCCAGGAA CCCAAAGGTG GTGGAGAACC ACTTCCAAGG
Thai-EU-1-N-terminal-Sn   GTAGTGAGCC ACTCCAGGAA CCCAAAGGTG GTGGAGAACC ACTTCCAAGG
Thai-US-1-N-terminal-Sn   GTAGTGAGCC ACTCCAGGAA CCCAAAGGTG GTGGAGAACC ACTTCCAAGG

                          ....|....| ....|....| ....|....| ....|....| ....|....|
                                 410        420        430        440        450
refSn                     CCGGGCCCTG CTGTTGGGGC AGGTTGAACA GAGGACGTGC AGCCTGCTGC
Thai-HP-1-N-terminal-Sn   CCGGGCCCTG CTGTTGGGGC AGGTTGAACA GAGGACGTGC AGCCTGCTGC
Thai-EU-1-N-terminal-Sn   CCGGGCCCTG CTGTTGGGGC AGGTTGAACA GAGGACGTGC AGCCTGCTGC
Thai-US-1-N-terminal-Sn   CCGGGCCCTG CTGTTGGGGC AGGTTGAACA GAGGACGTGC AGCCTGCTGC
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                                  460        470        480        490        500
refSn                     TGAAGGACCT GCAGCCCCAG GACTCGGGCT CCTATAACTT CCGCTTTGAG
Thai-HP-1-N-terminal-Sn   TGAAGGACCT GCAGCCCCAG GACTCGGGCT CCTATAACTT CCGCTTTGAG
Thai-EU-1-N-terminal-Sn   TGAAGGACCT GCAGCCCCAG GACTCGGGCT CCTATAACTT CCGCTTTGAG
Thai-US-1-N-terminal-Sn   TGAAGGACCT GCAGCCCCAG GACTCGGGCT CCTATAACTT CCGCTTTGAG

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                                  510        520        530        540        550
refSn                     ATCAGCGAGG GCAACCGCTG GTCAGATGTC AAAGGCACAG TTGTCACCGT
Thai-HP-1-N-terminal-Sn   ATCAGCGAGG GCAACCGCTG GTCAGATGTC AAAGGCACAG TTGTCACCGT
Thai-EU-1-N-terminal-Sn   ATCAGCGAGG GCAACCGCTG GTCAGATGTC AAAGGCACAG TTGTCACCGT
Thai-US-1-N-terminal-Sn   ATCAGCGAGG GCAACCGCTG GTCAGATGTC AAAGGCACAG TTGTCACCGT

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                                  560        570        580        590        600
refSn                     GACAGAGGTG CCCAGCGTGC CCACCATTGC CTTGCCAGCC AAGCTGCATG
Thai-HP-1-N-terminal-Sn   GACAGAGGTG CCCAGCGTGC CCACCATTGC CTTGCCAGCC AAGCTGCATG
Thai-EU-1-N-terminal-Sn   GACAGAGGTG CCCAGCGTGC CCACCATTGC CTTGCCAGCC AAGCTGCATG
Thai-US-1-N-terminal-Sn   GACAGAGGTG CCCAGCGTGC CCACCATTGC CTTGCCAGCC AAGCTGCATG

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                                  610        620        630        640        650
refSn                     AGGGCATGGA GGTGGACTTC AACTGCTCCA CTCCCTATGT GTGCCCGACG
Thai-HP-1-N-terminal-Sn   AGGGCATGGA GGTGGACTTC AACTGCTCCA CTCCCTATGT GTGCCCGACG
Thai-EU-1-N-terminal-Sn   AGGGCATGGA GGTGGACTTC AACTGCTCCA CTCCCTATGT GTGCCCGACG
Thai-US-1-N-terminal-Sn   AGGGCATGGA GGTGGACTTC AACTGCTCCA CTCCCTATGT GTGCCCGACG

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                                  660        670        680        690        700
refSn                     GAGCCGGTCA ACCTACAGTG GCAAGGCCAG GATCCCACCC GCTCCGTCAC
Thai-HP-1-N-terminal-Sn   GAGCCGGTCA ACCTACAGTG GCAAGGCCAG GATCCCACCC GCTCCGTCAC
Thai-EU-1-N-terminal-Sn   GAGCCGGTCA ACCTACAGTG GCAAGGCCAG GATCCCACCC GCTCCGTCAC
Thai-US-1-N-terminal-Sn   GAGCCGGTCA ACCTACAGTG GCAAGGCCAG GATCCCACCC GCTCCGTCAC

                          ....|....| ....|....| ....|....| ....|....| ....|....|
                                  710        720        730        740        750
refSn                     CTCCCACCTC CAGAAGCTTG AGCCCTCGGG CACCAGCCAC ATGGAGACCC
Thai-HP-1-N-terminal-Sn   CTCCCACCTC CAGAAGCTTG AGCCCTCGGG CACCAGCCAC ATGGAGACCC
Thai-EU-1-N-terminal-Sn   CTCCCACCTC CAGAAGCTTG AGCCCTCGGG CACCAGCCAC ATGGAGACCC
Thai-US-1-N-terminal-Sn   CTCCCACCTC CAGAAGCTTG AGCCCTCGGG CACCAGCCAC ATGGAGACCC

                          ....|....| ....|....| ....|....| ....|....| ....|....|
                                  760        770        780        790        800
refSn                     TGCACATGGC CCTGTCCTGG CAGGACCATG GCCGGATCCT GAGCTGCCAG
Thai-HP-1-N-terminal-Sn   TGCACATGGC CCTGTCCTGG CAGGACCATG GCCGGATCCT GAGCTGCCAG
Thai-EU-1-N-terminal-Sn   TGCACATGGC CCTGTCCTGG CAGGACCATG GCCGGATCCT GAGCTGCCAG
Thai-US-1-N-terminal-Sn   TGCACATGGC CCTGTCCTGG CAGGACCATG GCCGGATCCT GAGCTGCCAG

                          ....|....| ....|....| ....|....| ....|....| ....|....|
                                  810        820        830        840        850
refSn                     GTCTCAGCAG CCGAACGCAG GATGCAGAAG GAGATTCACC TCCAAGTGCA
Thai-HP-1-N-terminal-Sn   GTCTCAGCAG CCGAACGCAG GATGCAGAAG GAGATTCACC TCCAAGTGCA
Thai-EU-1-N-terminal-Sn   GTCTCAGCAG CCGAACGCAG GATGCAGAAG GAGATTCACC TCCAAGTGCA
Thai-US-1-N-terminal-Sn   GTCTCAGCAG CCGAACGCAG GATGCAGAAG GAGATTCACC TCCAAGTGCA

                          ....|....| ....|....| ....|....| ....|....| ....|....|
                                  860        870        880        890        900
refSn                     GTATGCCCCC AAGGGTGTGG AGATCCTTTT CAGCCACTCC GGACGGAACG
Thai-HP-1-N-terminal-Sn   GTATGCCCCC AAGGGTGTGG AGATCCTTTT CAGCCACTCC GGACGGAACG
Thai-EU-1-N-terminal-Sn   GTATGCCCCC AAGGGTGTGG AGATCCTTTT CAGCCACTCC GGACGGAACG
Thai-US-1-N-terminal-Sn   GTATGCCCCC AAGGGTGTGG AGATCCTTTT CAGCCACTCC GGACGGAACG
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                          ....|....| ....|....| ....|....| ....|....| ....|....|
                                  910        920        930        940        950
refSn                     TCCTTCCAGG TGATCTGGTC ACCCTCAGCT GCCAGGTGAA TAGCAGCAAC
Thai-HP-1-N-terminal-Sn   TCCTTCCTGG TGATCTGGTC ACCCTCAGCT GCCAGGTGAA TAGCAGCAAC
Thai-EU-1-N-terminal-Sn   TCCTTCCAGG TGATCTGGTC ACCCTCAGCT GCCAGGTGAA TAGCAGCAAC
Thai-US-1-N-terminal-Sn   TCCTTCCTGG TGATCTGGTC ACCCTCAGCT GCCAGGTGAA TAGCAGCAAC


                          ....|....| ....|....| ....|....| ....|....| ....|....|
                                 960        970        980        990       1000
refSn                     CCTCAGGTCA GTTCCGTGCA GTGGGTCAAG GATGGGACGA AGCTCAAAGA
Thai-HP-1-N-terminal-Sn   CCTCAGGTCA GTTCCGTGCA GTGGGTCAAG GATGGGACGA AGCTCAAAGA
Thai-EU-1-N-terminal-Sn   CCTCAGGTCA GTTCCGTGCA GTGGGTCAAG GATGGGACGA AGCTCAAAGA
Thai-US-1-N-terminal-Sn   CCTCAGGTCA GTTCCGTGCA GTGGGTCAAG GATGGGACGA AGCTCAAAGA

                          ....|....| ....|....| ....|....| ....|....| ....|....|
                                1010       1020       1030       1040       1050
refSn                     CCAGAAACGT GTACTGCAGT TGCGCCGGGC AGCCTGGGCT GATGCTGGCG
Thai-HP-1-N-terminal-Sn   CCAGAAACGT GTACTGCAGT TGCGCCGGGC AGCCTGGGCT GATGCTGGCG
Thai-EU-1-N-terminal-Sn   CCAGAAACGT GTACTGCAGT TGCGCCGGGC AGCCTGGGCT GATGCTGGCG
Thai-US-1-N-terminal-Sn   CCAGAAACGT GTACTGCAGT TGCGCCGGGC AGCCTGGGCT GATGCTGGCG

                          ....|....| ....|....| ....|....| ....|....| ....|....|
                                1060       1070       1080       1090       1100
refSn                     TCTACACCTG CCAAGCCGGG AATGCCGTGG GCTCTTCAGT CTCACCCCCG
Thai-HP-1-N-terminal-Sn   TCTACACCTG CCAAGCCGGG AATGCCGTGG GCTCTTCAGT CTCACCCCCG
Thai-EU-1-N-terminal-Sn   TCTACACCTG CCAAGCCGGG AATGCCGTGG GCTCTTCAGT CTCACCCCCG
Thai-US-1-N-terminal-Sn   TCTACACCTG CCAAGCCGGG AATGCCGTGG GCTCTTCAGT CTCACCCCCG

                          ....|....| ....|....| ....|....| ....|....| ....|....|
                                1110       1120       1130       1140       1150
refSn                     GTCAGCCTCC ACGTCTTCAT GGCTGAGGTC CAGGTAAGCC CTGTGGGCTC
Thai-HP-1-N-terminal-Sn   GTCAGCCTCC ACGTCTTCAT GGCTGAGGTC CAGGTAAGCC CTGTGGGCTC
Thai-EU-1-N-terminal-Sn   GTCAGCCTCC ACGTCTTCAT GGCTGAGGTC CAGGTAAGCC CTGTGGGCTC
Thai-US-1-N-terminal-Sn   GTCAGCCTCC ACGTCTTCAT GGCTGAGGTC CAGGTAAGCC CTGTGGGCTC

                          ....|....| ....|....|
                                1160       1170
refSn                     CATCCTGGAG AACCAGACGG
Thai-HP-1-N-terminal-Sn   CATCCTGGAG AACCAGACGG
Thai-EU-1-N-terminal-Sn   CATCCTGGAG AACCAGACGG
Thai-US-1-N-terminal-Sn   CATCCTGGAG AACCAGACGG
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Figure 4.11 Nucleotide sequence alignments of the reference Sn (refSn) (accession number is NM_214346) with recombinant plasmids of Thai-HP-1-pCR®8-GW-TOPO®-N-terminal Sn (Thai-HP-1-N-terminal-Sn), Thai-EU-1-pCR®8-GW-TOPO®-N-terminal Sn (Thai-EU-1-N-terminal-Sn) and Thai-US-1-pCR®8-GW-TOPO®-N-terminal Sn (Thai-US-1-N-terminal-Sn), respectively. The nucleotide changes are boxed.

Table 4.6 Deduced amino acid sequences of 3 recombinant porcine Sn (N-terminal domain) plasmids

Product's name	Sequence
1. Thai-HP-1-N-terminal-Sn (341 aa)	MDFLLLLLLLASSALAGLASWTVSSPETVQGIKGSCLIIPCTFGFPANVEVPH GITAIWYYDYSGKRLVVSHSRNPKVVENHFQGRALLLGQVEQRTCSLLLKDL QPQDSGSYNFRFEISEGNRWSDVKGTVVTVTEVPSVPTIALPAKLHEGMEVD FNCSTPYVCPTEPVNLQWQGQDPTRSVTSHLQKLEPSGTRHMETLHMALS WQDHGRILSCQVSAAERRMQKEIHLQVQHAPKGVEILFSHSGRNVLPGDLV TLSCQVNSSNPQVSSVQWVKDGTKLKDQKCVLQLRRAAWADAGVYTCQA GNAVGSSVSPPVSLHVFMAEVQVSPVGSILENQT
2. Thai-EU-1-N-terminal-Sn (341 aa)	MDFLLLLLLLASSALAGLASWTVSSPETVQGIKGSCLIIPCTFGFPANVEVPH GITAIWYYDYSGKRVVVSHSRNPKVVENHFQGRALLLGQVEQRTCSLLLKDL QPQDSGSYNFRFEISEGNRWSDVKGTVVTVTEVPSVPTIALPAKLHEGMEVD FNCSTPYVCPTEPVNLQWQGQYPTRSVTSHLQKLEPSGTRHMETLHMALS WQDHGRILSCQVSAAERRMQKEIHLQVQHAPKGVEILFSHSGRNVLPGDLV TLSCQVNSSNPQVSSVQWVKDGTKLKDQKRVLQLRRAAWADAGVYTCQA GNAVGSSVSPPVSLHVFMAEVQVSPVGSILENQT
3. Thai-US-1-N-terminal-Sn (341 aa)	MDFLLLLLLLASSALAGLASWTVSSPETVQGIKGSCLIIPCTFGFPANVEVPH GITAIWYYDYSGKRLVVSHSRNPKVVENHFQGRALLLGQVEQRTCSLLLKDL QPQDSGSYNFRFEISEGNRWSDVKGTVVTVTEVPSVPTIALPAKLHEGMEVD FNCSTPYVCPTEPVNLQWQGQDPTRSVTSHLQKLEPSGTRHMETLHMALS WQDHGRILSCQVSAAERRMQKEIHLQVQHAPKGVEILFSHSGRNVLPGDLV TLSCQVNSSNPQVSSVQWVKDGTKLKDQKCVLQLRRAAWADAGVYTCQA GNAVGSSVSPPVSLHVFMAEVQVSPVGSILENQT

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                        ....|....| ....|....| ....|....| ....|....| ....|....|
                                10         20         30         40         50
refSn                    MDFLLLLLLL ASSALAGLAS WTVSSPETVQ GIKGSCLIIP CTFGFPANVE
Thai-HP-1-N-terminal-Sn  MDFLLLLLLL ASSALAGLAS WTVSSPETVQ GIKGSCLIIP CTFRFPANVE
Thai-EU-1-N-terminal-Sn  MDFLLLLLLL ASSALAGLAS WTVSSPETVQ GIKGSCLIIP CTFGFPANVE
Thai-US-1-N-terminal-Sn  MDFLLLLLLL ASSALAGLAS WTVSSPETVQ GIKGSCLIIP CTFRFPANVE

                         ....|....| ....|....| ....|....| ....|....| ....|....|
                                60         70         80         90        100
refSn                    VPHGITAIWY YDYSGKRLVV SHSRNPKVVE NHFQGRALLL GQVEQRTCSL
Thai-HP-1-N-terminal-Sn  VPHGITAIWY YDYSGKRLVV SHSRNPKVVE NHFQGRALLL GQVEQRTCSL
Thai-EU-1-N-terminal-Sn  VPHGITAIWY YDYSGKRLVV SHSRNPKVVE NHFQGRALLL GQVEQRTCSL
Thai-US-1-N-terminal-Sn  VPHGITAIWY YDYSGKRLVV SHSRNPKVVE NHFQGRALLL GQVEQRTCSL

                         ....|....| ....|....| ....|....| ....|....| ....|....|
                                110        120        130        140        150
refSn                    LLKDLQPQDS GSYNFRFEIS EGNRWSDVKG TVVTVTEVPS VPTIALPAKL
Thai-HP-1-N-terminal-Sn  LLKDLQPQDS GSYNFRFEIS EGNRWSDVKG TVVTVTEVPS VPTIALPAKL
Thai-EU-1-N-terminal-Sn  LLKDLQPQDS GSYNFRFEIS EGNRWSDVKG TVVTVTEVPS VPTIALPAKL
Thai-US-1-N-terminal-Sn  LLKDLQPQDS GSYNFRFEIS EGNRWSDVKG TVVTVTEVPS VPTIALPAKL

                         ....|....| ....|....| ....|....| ....|....| ....|....|
                                160        170        180        190        200
refSn                    HEGMEVDFNC STPYVCPTEP VNLQWQGQDP TRSVTSHLQK LEPSGTSHME
Thai-HP-1-N-terminal-Sn  HEGMEVDFNC STPYVCPTEP VNLQWQGQDP TRSVTSHLQK LEPSGTSHME
Thai-EU-1-N-terminal-Sn  HEGMEVDFNC STPYVCPTEP VNLQWQGQDP TRSVTSHLQK LEPSGTSHME
Thai-US-1-N-terminal-Sn  HEGMEVDFNC STPYVCPTEP VNLQWQGQDP TRSVTSHLQK LEPSGTSHME

                         ....|....| ....|....| ....|....| ....|....| ....|....|
                                210        220        230        240        250
refSn                    TLHMALSWQD HGRILSCQVS AAERRMQKEI HLQVQYAPKG VEILFSHSGR
Thai-HP-1-N-terminal-Sn  TLHMALSWQD HGRILSCQVS AAERRMQKEI HLQVQYAPKG VEILFSHSGR
Thai-EU-1-N-terminal-Sn  TLHMALSWQD HGRILSCQVS AAERRMQKEI HLQVQYAPKG VEILFSHSGR
Thai-US-1-N-terminal-Sn  TLHMALSWQD HGRILSCQVS AAERRMQKEI HLQVQYAPKG VEILFSHSGR

                         ....|....| ....|....| ....|....| ....|....| ....|....|
                                260        270        280        290        300
refSn                    NVLPGDLVTL SCQVNSSNPQ VSSVQWVKDG TKLKDQKRVL QLRRAAWADA
Thai-HP-1-N-terminal-Sn  NVLPGDLVTL SCQVNSSNPQ VSSVQWVKDG TKLKDQKRVL QLRRAAWADA
Thai-EU-1-N-terminal-Sn  NVLPGDLVTL SCQVNSSNPQ VSSVQWVKDG TKLKDQKRVL QLRRAAWADA
Thai-US-1-N-terminal-Sn  NVLPGDLVTL SCQVNSSNPQ VSSVQWVKDG TKLKDQKRVL QLRRAAWADA

                         ....|....| ....|....| ....|....| ....|....| .
                                310        320        330        340
refSn                    GVYTCQAGNA VGSSVSPPVS LHVFMAEVQV SPVGSILENQ T
Thai-HP-1-N-terminal-Sn  GVYTCQAGNA VGSSVSPPVS LHVFMAEVQV SPVGSILENQ T
Thai-EU-1-N-terminal-Sn  GVYTCQAGNA VGSSVSPPVS LHVFMAEVQV SPVGSILENQ T
Thai-US-1-N-terminal-Sn  GVYTCQAGNA VGSSVSPPVS LHVFMAEVQV SPVGSILENQ T
```

Figure 4.12 Deduced amino acid alignments of the reference Sn (refSn) (GenBank accession number is NP_999511) with recombinant plasmids of Thai-HP-1-pCR®8-GW-TOPO®-N-terminal Sn (Thai-HP-1-N-terminal-Sn), Thai-EU-1-pCR®8-GW-TOPO®-N-terminal Sn (Thai-EU-1-N-terminal-Sn) and Thai-US-1-pCR®8-GW-TOPO®-N-terminal Sn (Thai-US-1-N-terminal-Sn), respectively. The amino acid change is boxed.

2.3 Nucleotide and deduced amino acid sequences of recombinant porcine CD163 (full) plasmids

The author selected 3 recombinant plasmids of Thai-HP-1-pCR®-XL-TOPO®-CD163, (Thai-HP-1-CD163), Thai-EU-1-pCR®-XL-TOPO®-CD163 (Thai-EU-1-CD163) and Thai-US-1-pCR®-XL-TOPO®-CD163 (Thai-US-1-CD163) to sequence. The 3 recombinant porcine CD163 (full) plasmids were successfully sequenced (3,400 bp) and were analyzed alignment with the available data bases of the reference CD163 (refCD163) cDNA (accession number is NM_213976) form the PAM of normal pigs as shown in Table 4.7 and Figure 4.13, respectively. The results revealed that the nucleotide sequences of 3 recombinant porcine CD163 (full) plasmids were showed similar pattern and changed in 11 positions at 796; 960; 1,370; 1,965; 2,437; 2,455; 2,700; 2,798; 2,897; 2,898 and 3,240, respectively.

The deduced amino acid sequences and alignment of 3 recombinant porcine CD163 (full) plasmids from PRRVS infected PAM (1,133 amino acids) and the refCD163 (accession number is NP_999141) from normal PAM (1,133 amino acids) were shown in Table 4.8 and Figure 4.14, respectively. The results showed that the amino acid sequences of 3 recombinant porcine CD163 (full) plasmids were changed in 6 positions at 266 (Leucine to Valine); 457 (Aspartate to Alanine); 655 (Aspartate to Glutamate); 813 (Serine to Proline); 819 (Arginine to Glycine) and 933 (Serine to Phenylalanine), respectively.

Table 4.7 Nucleotide sequences of 3 recombinant porcine CD163 (full) plasmids

Transformant's name	Sequence
1. Thai-HP-1-CD163 (3,400 bp)	atggtgctacttgaagactctggatctgcagactttagaagatgttctgcccatttaagttccttcactt ttgctgtagtcgctgttctcagtgcctgcttggtcactagttctcttggaggaaaagacaaggagct gaggctaacgggtggtgaaaacaagtgctctggaagagtggaggtgaaagtgcaggagga gtggggaactgtgtgtaataatggctgggacatggatgtggtctctgttgtttgtaggcagctggg atgtccaactgctatcaaagccactggatgggctaattttagtgcaggttctggacgcatttggatg gatcatgtttcttgtcgagggaatgagtcagctctctgggactgcaaacatgatggatggggaaa gcataactgtactcaccaacaggatgctggagtaacctgctcagatggatctgatttagagatga ggctggtgaatggaggaaaccggtgcttaggaagaatagaagtcaaatttcaagagcggtgg ggaacagtgtgtgatgataacttcaacataaatcatgcttctgtggtttgtaaacaacttgaatgtgg aagtgctgtcagtttctctggttcagctaattttggagaaggttctggaccaatctggtttgatgatctt gtatgcaatggaaatgagtcagctctctggaactgcaaacatgaaggatggggaaagcacaat tgcgatcatgctgaggatgctggagtgatttgcttaaatggagcagacctgaaactgagagtggt agatggagtcactgaatgttcaggaagattggaagtgaaattccaaggagaatggggaacaat ctgtgatgatggctgggatagtgatgatgccgctgtggcatgtaagcaactgggatgtccaactg ctgtcactgccattggtcgagttaacgccagtgagggaactggtcacatttggcttgacagtgtttc ttgccatggacacgagtctgctctctggcagtgtagacaccatgaatggggaaagcattattgca atcataatgaagatgctggtgtgacatgttctgatggatcagatctggaactgagacttaaaggtg gaggcagccactgtgctgggacagtggaggtggaaattcagaaactggtaggaaaagtgtgt gatagaagctggggactgaaagaagctgatgtggtttgcaggcagctgggatgtggatctgca ctcaaaacatcatatcaagtttattccaaaaccaaggcaacaaacacatggctgtttgtaagcag ctgtaatggaaatgaaacttctctttgggactgcaagaattggcagtggggtggacttagttgtgct cactatgacgaagccaaaattacctgctcagcccacaggaaacccaggctggttggagggg acattccctgctctggtcgtgttgaagtacaacatggagacacgtggggcaccgtctgtgattctg acttctctctggaggcggccagcgtgctgtgcagggaactacagtgcggcactgtggtttccctc

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	gatatgctaaatccctcaggagaccactctgaagtacaatgaaaaggaaaatgggaattataac ctggtgagttcagccttgatgaagacctggacta
2. Thai-EU-1-CD163 (3,400 bp)	atggtgctacttgaagactctggatctgcagactttagaagatgttctgcccatttaagttccttcactt ttgctgtagtcgctgttctcagtgcctgcttggtcactagttctcttggaggaaaagacaaggagct gaggctaacgggtggtgaaaacaagtgctctggaagagtggaggtgaaagtgcaggagga gtggggaactgtgtgtaataatggctgggacatggatgtggtctctgttgtttgtaggcagctggg atgtccaactgctatcaaagccactggatgggctaattttagtgcaggttctggacgcatttggatg gatcatgtttcttgtcgagggaatgagtcagctctctgggactgcaaacatgatggatggggaaa gcataactgtactcaccaacaggatgctggagtaacctgctcagatggatctgatttagagatga ggctggtgaatggaggaaaccggtgcttaggaagaatagaagtcaaatttcaagagcggtgg ggaacagtgtgtgatgataacttcaacataaatcatgcttctgtggtttgtaaacaacttgaatgtgg aagtgctgtcagtttctctggttcagctaattttggagaaggttctggaccaatctggtttgatgatctt gtatgcaatggaaatgagtcagctctctggaactgcaaacatgaaggatggggaaagcacaat tgcgatcatgctgaggatgctggagtgatttgcttaaatggagcagacctgaaactgagagtggt agatggagtcactgaatgttcaggaagattggaagtgaaattccaaggagaatggggaacaat ctgtgatgatggctgggatagtgatgatgccgctgtggcatgtaagcaactgggatgtccaactg ctgtcactgccattggtcgagttaacgccagtgagggaactggtcacatttggcttgacagtgtttc ttgccatggacacgagtctgctctctggcagtgtagacaccatgaatggggaaagcattattgca atcataatgaagatgctggtgtgacatgttctgatggatcagatctggaactgagacttaaaggtg gaggcagccactgtgctgggacagtggaggtggaaattcagaaactggtaggaaaagtgtgt gatagaagctggggactgaaagaagctgatgtggtttgcaggcagctgggatgtggatctgca ctcaaaacatcatatcaagtttattccaaaaccaaggcaacaaacacatggctgtttgtaagcag ctgtaatggaaatgaaacttctctttgggactgcaagaattggcagtggggtggacttagttgtgat cactatgacgaagccaaaattacctgctcagcccacaggaaacccaggctggttggagggg acattccctgctctggtcgtgttgaagtacaacatggagacacgtggggcaccgtctgtgattctg acttctctctggaggcggccagcgtgctgtgcagggaactacagtgcggcactgtggtttccctc ctgggggggagctcactttggagaaggaagtggacagatctgggctgaagaattccagtgtgag

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	cctggtgagttcagccttgatgaagacctggacta
3. Thai-US-1-CD163 (3,400 bp)	atggtgctacttgaagactctggatctgcagactttagaagatgttctgcccatttaagttccttcactt ttgctgtagtcgctgttctcagtgcctgcttggtcactagttctcttggaggaaaagacaaggagct gaggctaacgggtggtgaaaacaagtgctctggaagagtggaggtgaaagtgcaggagga gtggggaactgtgtgtaataatggctgggacatggatgtggtctctgttgtttgtaggcagctggg atgtccaactgctatcaaagccactggatgggctaattttagtgcaggttctggacgcatttggatg gatcatgtttcttgtcgagggaatgagtcagctctctgggactgcaaacatgatggatggggaaa gcataactgtactcaccaacaggatgctggagtaacctgctcagatggatctgatttagagatga ggctggtgaatggaggaaaccggtgcttaggaagaatagaagtcaaatttcaagagcggtgg ggaacagtgtgtgatgataacttcaacataaatcatgcttctgtggtttgtaaacaacttgaatgtgg aagtgctgtcagtttctctggttcagctaattttggagaaggttctggaccaatctggtttgatgatctt gtatgcaatggaaatgagtcagctctctggaactgcaaacatgaaggatggggaaagcacaat tgcgatcatgctgaggatgctggagtgatttgcttaaatggagcagacctgaaactgagagtggt agatggagtcactgaatgttcaggaagattggaagtgaaattccaaggagaatggggaacaat ctgtgatgatggctgggatagtgatgatgccgctgtggcatgtaagcaactgggatgtccaactg ctgtcactgccattggtcgagttaacgccagtgagggaactggtcacatttggcttgacagtgtttc ttgccatggacacgagtctgctctctggcagtgtagacaccatgaatggggaaagcattattgca atcataatgaagatgctggtgtgacatgttctgatggatcagatctggaactgagacttaaaggtg gaggcagccactgtgctgggacagtggaggtggaaattcagaaactggtaggaaaagtgtgt gatagaagctggggactgaaagaagctgatgtggtttgcaggcagctgggatgtggatctgca ctcaaaacatcatatcaagtttattccaaaaccaaggcaacaaacacatggctgtttgtaagcag ctgtaatggaaatgaaacttctctttgggactgcaagaattggcagtggggtggacttagttgtgct cactatgacgaagccaaaattacctgctcagcccacaggaaacccaggctggttggagggg acattccctgctctggtcgtgttgaagtacaacatggagacacgtggggcaccgtctgtgattctg acttctctctggaggcggccagcgtgctgtgcagggaactacagtgcggcactgtggtttccctc ctgggggggagctcactttggagaaggaagtggacagatctgggctgaagaattccagtgtgag gggcacgagtcccacctttcactctgcccagtagcaccccgccctgacgggacatgtagcca

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gactgtggacacaaggaggatgctgctgtgacgtgctcagaaattgcaaagagccgagaatc
cctacatgccacaggtcgctcatcttttgttgcacttgcaatctttggggtcattctgttggcctgtctc
atcgcattcctcatttggactcagaagcgaagacagaggcagcggctctcagttttctcaggag
gagagaattctgtacatcaaattcaataccgggagatgaattcttgcctgaaagcagatgaaacg
gatatgctaaatccctcaggagaccactctgaagtacaatgaaaaggaaaatgggaattataac
ctggtgagttcagccttgatgaagacctggacta

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                    ....|....| ....|....| ....|....| ....|....| ....|....|
                         10         20         30         40         50
                    M13 reverse
refCD163            ---------- ---------- ---------- ---------- ----------
Thai-HP-1-CD163     CAGGAAACAG CTATGACCAT GATTACGCCA AGCTATTTAG GTGACGCGTT
Thai-EU-1-CD163     CAGGAAACAG CTATGACCAT GATTACGCCA AGCTATTTAG GTGACGCGTT
Thai-US-1-CD163     CAGGAAACAG CTATGACCAT GATTACGCCA AGCTATTTAG GTGACGCGTT

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                         60         70         80         90        100
refCD163            ---------- ---------- ---------- ---------- ----------
Thai-HP-1-CD163     AGAATACTCA AGCTATGCAT CAAGCTTGGT ACCGAGCTCG GATCCACTAG
Thai-EU-1-CD163     AGAATACTCA AGCTATGCAT CAAGCTTGGT ACCGAGCTCG GATCCACTAG
Thai-US-1-CD163     AGAATACTCA AGCTATGCAT CAAGCTTGGT ACCGAGCTCG GATCCACTAG

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                        110        120        130        140        150
refCD163            ---------- ---------- ---------- --ATGGTGCT ACTTGAAGAC
Thai-HP-1-CD163     TAACGGCCGC CAGTGTGCTG GAATTCGCCC TTATGGTGCT ACTTGAAGAC
Thai-EU-1-CD163     TAACGGCCGC CAGTGTGCTG GAATTCGCCC TTATGGTGCT ACTTGAAGAC
Thai-US-1-CD163     TAACGGCCGC CAGTGTGCTG GAATTCGCCC TTATGGTGCT ACTTGAAGAC

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                        160        170        180        190        200
refCD163            TCTGGATCTG CAGACTTTAG AAGATGTTCT GCCCATTTAA GTTCCTTCAC
Thai-HP-1-CD163     TCTGGATCTG CAGACTTTAG AAGATGTTCT GCCCATTTAA GTTCCTTCAC
Thai-EU-1-CD163     TCTGGATCTG CAGACTTTAG AAGATGTTCT GCCCATTTAA GTTCCTTCAC
Thai-US-1-CD163     TCTGGATCTG CAGACTTTAG AAGATGTTCT GCCCATTTAA GTTCCTTCAC

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                        210        220        230        240        250
refCD163            TTTTGCTGTA GTCGCTGTTC TCAGTGCCTG CTTGGTCACT AGTTCTCTTG
Thai-HP-1-CD163     TTTTGCTGTA GTCGCTGTTC TCAGTGCCTG CTTGGTCACT AGTTCTCTTG
Thai-EU-1-CD163     TTTTGCTGTA GTCGCTGTTC TCAGTGCCTG CTTGGTCACT AGTTCTCTTG
Thai-US-1-CD163     TTTTGCTGTA GTCGCTGTTC TCAGTGCCTG CTTGGTCACT AGTTCTCTTG

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                        260        270        280        290        300
refCD163            GAGGAAAAGA CAAGGAGCTG AGGCTAACGG GTGGTGAAAA CAAGTGCTCT
Thai-HP-1-CD163     GAGGAAAAGA CAAGGAGCTG AGGCTAACGG GTGGTGAAAA CAAGTGCTCT
Thai-EU-1-CD163     GAGGAAAAGA CAAGGAGCTG AGGCTAACGG GTGGTGAAAA CAAGTGCTCT
Thai-US-1-CD163     GAGGAAAAGA CAAGGAGCTG AGGCTAACGG GTGGTGAAAA CAAGTGCTCT

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                        310        320        330        340        350
refCD163            GGAAGAGTGG AGGTGAAAGT GCAGGAGGAG TGGGGAACTG TGTGTAATAA
Thai-HP-1-CD163     GGAAGAGTGG AGGTGAAAGT GCAGGAGGAG TGGGGAACTG TGTGTAATAA
Thai-EU-1-CD163     GGAAGAGTGG AGGTGAAAGT GCAGGAGGAG TGGGGAACTG TGTGTAATAA
Thai-US-1-CD163     GGAAGAGTGG AGGTGAAAGT GCAGGAGGAG TGGGGAACTG TGTGTAATAA

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                        360        370        380        390        400
refCD163            TGGCTGGGAC ATGGATGTGG TCTCTGTTGT TTGTAGGCAG CTGGGATGTC
Thai-HP-1-CD163     TGGCTGGGAC ATGGATGTGG TCTCTGTTGT TTGTAGGCAG CTGGGATGTC
Thai-EU-1-CD163     TGGCTGGGAC ATGGATGTGG TCTCTGTTGT TTGTAGGCAG CTGGGATGTC
Thai-US-1-CD163     TGGCTGGGAC ATGGATGTGG TCTCTGTTGT TTGTAGGCAG CTGGGATGTC
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                             410        420        430        440        450
refCD163            CAACTGCTAT CAAAGCCACT GGATGGGCTA ATTTTAGTGC AGGTTCTGGA
Thai-HP-1-CD163     CAACTGCTAT CAAAGCCACT GGATGGGCTA ATTTTAGTGC AGGTTCTGGA
Thai-EU-1-CD163     CAACTGCTAT CAAAGCCACT GGATGGGCTA ATTTTAGTGC AGGTTCTGGA
Thai-US-1-CD163     CAACTGCTAT CAAAGCCACT GGATGGGCTA ATTTTAGTGC AGGTTCTGGA

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                             460        470        480        490        500
refCD163            CGCATTTGGA TGGATCATGT TTCTTGTCGA GGGAATGAGT CAGCTCTCTG
Thai-HP-1-CD163     CGCATTTGGA TGGATCATGT TTCTTGTCGA GGGAATGAGT CAGCTCTCTG
Thai-EU-1-CD163     CGCATTTGGA TGGATCATGT TTCTTGTCGA GGGAATGAGT CAGCTCTCTG
Thai-US-1-CD163     CGCATTTGGA TGGATCATGT TTCTTGTCGA GGGAATGAGT CAGCTCTCTG

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                             510        520        530        540        550
refCD163            GGACTGCAAA CATGATGGAT GGGGAAAGCA TAACTGTACT CACCAACAGG
Thai-HP-1-CD163     GGACTGCAAA CATGATGGAT GGGGAAAGCA TAACTGTACT CACCAACAGG
Thai-EU-1-CD163     GGACTGCAAA CATGATGGAT GGGGAAAGCA TAACTGTACT CACCAACAGG
Thai-US-1-CD163     GGACTGCAAA CATGATGGAT GGGGAAAGCA TAACTGTACT CACCAACAGG

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                             560        570        580        590        600
refCD163            ATGCTGGAGT AACCTGCTCA GATGGATCTG ATTTAGAGAT GAGGCTGGTG
Thai-HP-1-CD163     ATGCTGGAGT AACCTGCTCA GATGGATCTG ATTTAGAGAT GAGGCTGGTG
Thai-EU-1-CD163     ATGCTGGAGT AACCTGCTCA GATGGATCTG ATTTAGAGAT GAGGCTGGTG
Thai-US-1-CD163     ATGCTGGAGT AACCTGCTCA GATGGATCTG ATTTAGAGAT GAGGCTGGTG

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                             610        620        630        640        650
refCD163            AATGGAGGAA ACCGGTGCTT AGGAAGAATA GAAGTCAAAT TTCAAGAGCG
Thai-HP-1-CD163     AATGGAGGAA ACCGGTGCTT AGGAAGAATA GAAGTCAAAT TTCAAGAGCG
Thai-EU-1-CD163     AATGGAGGAA ACCGGTGCTT AGGAAGAATA GAAGTCAAAT TTCAAGAGCG
Thai-US-1-CD163     AATGGAGGAA ACCGGTGCTT AGGAAGAATA GAAGTCAAAT TTCAAGAGCG

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                             660        670        680        690        700
refCD163            GTGGGGAACA GTGTGTGATG ATAACTTCAA CATAAATCAT GCTTCTGTGG
Thai-HP-1-CD163     GTGGGGAACA GTGTGTGATG ATAACTTCAA CATAAATCAT GCTTCTGTGG
Thai-EU-1-CD163     GTGGGGAACA GTGTGTGATG ATAACTTCAA CATAAATCAT GCTTCTGTGG
Thai-US-1-CD163     GTGGGGAACA GTGTGTGATG ATAACTTCAA CATAAATCAT GCTTCTGTGG

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                             710        720        730        740        750
refCD163            TTTGTAAACA ACTTGAATGT GGAAGTGCTG TCAGTTTCTC TGGTTCAGCT
Thai-HP-1-CD163     TTTGTAAACA ACTTGAATGT GGAAGTGCTG TCAGTTTCTC TGGTTCAGCT
Thai-EU-1-CD163     TTTGTAAACA ACTTGAATGT GGAAGTGCTG TCAGTTTCTC TGGTTCAGCT
Thai-US-1-CD163     TTTGTAAACA ACTTGAATGT GGAAGTGCTG TCAGTTTCTC TGGTTCAGCT

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                             760        770        780        790        800
refCD163            AATTTTGGAG AAGGTTCTGG ACCAATCTGG TTTGATGATC TTGTATGCAA
Thai-HP-1-CD163     AATTTTGGAG AAGGTTCTGG ACCAATCTGG TTTGATGATC TTGTATGCAA
Thai-EU-1-CD163     AATTTTGGAG AAGGTTCTGG ACCAATCTGG TTTGATGATC TTGTATGCAA
Thai-US-1-CD163     AATTTTGGAG AAGGTTCTGG ACCAATCTGG TTTGATGATC TTGTATGCAA
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                             810        820        830        840        850
refCD163            TGGAAATGAG TCAGCTCTCT GGAACTGCAA ACATGAAGGA TGGGGAAAGC
Thai-HP-1-CD163     TGGAAATGAG TCAGCTCTCT GGAACTGCAA ACATGAAGGA TGGGGAAAGC
Thai-EU-1-CD163     TGGAAATGAG TCAGCTCTCT GGAACTGCAA ACATGAAGGA TGGGGAAAGC
Thai-US-1-CD163     TGGAAATGAG TCAGCTCTCT GGAACTGCAA ACATGAAGGA TGGGGAAAGC

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                             860        870        880        890        900
refCD163            ACAATTGCGA TCATGCTGAG GATGCTGGAG TGATTTGCTT AAATGGAGCA
Thai-HP-1-CD163     ACAATTGCGA TCATGCTGAG GATGCTGGAG TGATTTGCTT AAATGGAGCA
Thai-EU-1-CD163     ACAATTGCGA TCATGCTGAG GATGCTGGAG TGATTTGCTT AAATGGAGCA
Thai-US-1-CD163     ACAATTGCGA TCATGCTGAG GATGCTGGAG TGATTTGCTT AAATGGAGCA

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                             910        920        930        940        950
refCD163            GACCTGAAAC TGAGAGTGGT AGATGGACTC ACTGAATGTT CAGGAAGATT
Thai-HP-1-CD163     GACCTGAAAC TGAGAGTGGT AGATGGAGTC ACTGAATGTT CAGGAAGATT
Thai-EU-1-CD163     GACCTGAAAC TGAGAGTGGT AGATGGAGTC ACTGAATGTT CAGGAAGATT
Thai-US-1-CD163     GACCTGAAAC TGAGAGTGGT AGATGGAGTC ACTGAATGTT CAGGAAGATT

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                            960        970        980        990       1000
refCD163            GGAAGTGAAA TTCCAAGGAG AATGGGGAAC AATCTGTGAT GATGGCTGGG
Thai-HP-1-CD163     GGAAGTGAAA TTCCAAGGAG AATGGGGAAC AATCTGTGAT GATGGCTGGG
Thai-EU-1-CD163     GGAAGTGAAA TTCCAAGGAG AATGGGGAAC AATCTGTGAT GATGGCTGGG
Thai-US-1-CD163     GGAAGTGAAA TTCCAAGGAG AATGGGGAAC AATCTGTGAT GATGGCTGGG

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                            1010       1020       1030       1040       1050
refCD163            ATAGTGATGA TGCCGCTGTG GCATGTAAGC AACTGGGATG TCCAACTGCT
Thai-HP-1-CD163     ATAGTGATGA TGCCGCTGTG GCATGTAAGC AACTGGGATG TCCAACTGCT
Thai-EU-1-CD163     ATAGTGATGA TGCCGCTGTG GCATGTAAGC AACTGGGATG TCCAACTGCT
Thai-US-1-CD163     ATAGTGATGA TGCCGCTGTG GCATGTAAGC AACTGGGATG TCCAACTGCT

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                            1060       1070       1080       1090       1100
refCD163            GTCACTGCCA TTGGTCGAGT TAACGCCAGT GAGGGAACTG GACACATTTG
Thai-HP-1-CD163     GTCACTGCCA TTGGTCGAGT TAACGCCAGT GAGGGAACTG GTCACATTTG
Thai-EU-1-CD163     GTCACTGCCA TTGGTCGAGT TAACGCCAGT GAGGGAACTG GTCACATTTG
Thai-US-1-CD163     GTCACTGCCA TTGGTCGAGT TAACGCCAGT GAGGGAACTG GTCACATTTG

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                            1110       1120       1130       1140       1150
refCD163            GCTTGACAGT GTTTCTTGCC ATGGACACGA GTCTGCTCTC TGGCAGTGTA
Thai-HP-1-CD163     GCTTGACAGT GTTTCTTGCC ATGGACACGA GTCTGCTCTC TGGCAGTGTA
Thai-EU-1-CD163     GCTTGACAGT GTTTCTTGCC ATGGACACGA GTCTGCTCTC TGGCAGTGTA
Thai-US-1-CD163     GCTTGACAGT GTTTCTTGCC ATGGACACGA GTCTGCTCTC TGGCAGTGTA

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                            1160       1170       1180       1190       1200
refCD163            GACACCATGA ATGGGGAAAG CATTATTGCA ATCATAATGA AGATGCTGGT
Thai-HP-1-CD163     GACACCATGA ATGGGGAAAG CATTATTGCA ATCATAATGA AGATGCTGGT
Thai-EU-1-CD163     GACACCATGA ATGGGGAAAG CATTATTGCA ATCATAATGA AGATGCTGGT
Thai-US-1-CD163     GACACCATGA ATGGGGAAAG CATTATTGCA ATCATAATGA AGATGCTGGT
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                            1210       1220       1230       1240       1250
refCD163            GTGACATGTT CTGATGGATC AGATCTGGAA CTGAGACTTA AAGGTGGAGG
Thai-HP-1-CD163     GTGACATGTT CTGATGGATC AGATCTGGAA CTGAGACTTA AAGGTGGAGG
Thai-EU-1-CD163     GTGACATGTT CTGATGGATC AGATCTGGAA CTGAGACTTA AAGGTGGAGG
Thai-US-1-CD163     GTGACATGTT CTGATGGATC AGATCTGGAA CTGAGACTTA AAGGTGGAGG

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                            1260       1270       1280       1290       1300
refCD163            CAGCCACTGT GCTGGGACAG TGGAGGTGGA AATTCAGAAA CTGGTAGGAA
Thai-HP-1-CD163     CAGCCACTGT GCTGGGACAG TGGAGGTGGA AATTCAGAAA CTGGTAGGAA
Thai-EU-1-CD163     CAGCCACTGT GCTGGGACAG TGGAGGTGGA AATTCAGAAA CTGGTAGGAA
Thai-US-1-CD163     CAGCCACTGT GCTGGGACAG TGGAGGTGGA AATTCAGAAA CTGGTAGGAA

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                            1310       1320       1330       1340       1350
refCD163            AAGTGTGTGA TAGAAGCTGG GGACTGAAAG AAGCTGATGT GGTTTGCAGG
Thai-HP-1-CD163     AAGTGTGTGA TAGAAGCTGG GGACTGAAAG AAGCTGATGT GGTTTGCAGG
Thai-EU-1-CD163     AAGTGTGTGA TAGAAGCTGG GGACTGAAAG AAGCTGATGT GGTTTGCAGG
Thai-US-1-CD163     AAGTGTGTGA TAGAAGCTGG GGACTGAAAG AAGCTGATGT GGTTTGCAGG

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                            1360       1370       1380       1390       1400
refCD163            CAGCTGGGAT GTGGATCTGC ACTCAAAACA TCATATCAAG TTTATTCCAA
Thai-HP-1-CD163     CAGCTGGGAT GTGGATCTGC ACTCAAAACA TCATATCAAG TTTATTCCAA
Thai-EU-1-CD163     CAGCTGGGAT GTGGATCTGC ACTCAAAACA TCATATCAAG TTTATTCCAA
Thai-US-1-CD163     CAGCTGGGAT GTGGATCTGC ACTCAAAACA TCATATCAAG TTTATTCCAA

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                            1410       1420       1430       1440       1450
refCD163            AACCAAGGCA ACAAACACAT GGCTGTTTGT AAGCAGCTGT AATGGAAATG
Thai-HP-1-CD163     AACCAAGGCA ACAAACACAT GGCTGTTTGT AAGCAGCTGT AATGGAAATG
Thai-EU-1-CD163     AACCAAGGCA ACAAACACAT GGCTGTTTGT AAGCAGCTGT AATGGAAATG
Thai-US-1-CD163     AACCAAGGCA ACAAACACAT GGCTGTTTGT AAGCAGCTGT AATGGAAATG

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                            1460       1470       1480       1490       1500
refCD163            AAACTTCTCT TTGGGACTGC AAGAATTGGC AGTGGGGTGG ACTTAGTTGT
Thai-HP-1-CD163     AAACTTCTCT TTGGGACTGC AAGAATTGGC AGTGGGGTGG ACTTAGTTGT
Thai-EU-1-CD163     AAACTTCTCT TTGGGACTGC AAGAATTGGC AGTGGGGTGG ACTTAGTTGT
Thai-US-1-CD163     AAACTTCTCT TTGGGACTGC AAGAATTGGC AGTGGGGTGG ACTTAGTTGT

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                            1510       1520       1530       1540       1550
refCD163            GATCACTATG ACGAAGCCAA AATTACCTGC TCAGCCCACA GGAAACCCAG
Thai-HP-1-CD163     GCTCACTATG ACGAAGCCAA AATTACCTGC TCAGCCCACA GGAAACCCAG
Thai-EU-1-CD163     GATCACTATG ACGAAGCCAA AATTACCTGC TCAGCCCACA GGAAACCCAG
Thai-US-1-CD163     GCTCACTATG ACGAAGCCAA AATTACCTGC TCAGCCCACA GGAAACCCAG

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                            1560       1570       1580       1590       1600
refCD163            GCTGGTTGGA GGGGACATTC CCTGCTCTGG TCGTGTTGAA GTACAACATG
Thai-HP-1-CD163     GCTGGTTGGA GGGGACATTC CCTGCTCTGG TCGTGTTGAA GTACAACATG
Thai-EU-1-CD163     GCTGGTTGGA GGGGACATTC CCTGCTCTGG TCGTGTTGAA GTACAACATG
Thai-US-1-CD163     GCTGGTTGGA GGGGACATTC CCTGCTCTGG TCGTGTTGAA GTACAACATG
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                            1610       1620       1630       1640       1650
refCD163            GAGACACGTG GGGCACCGTC TGTGATTCTG ACTTCTCTCT GGAGGCGGCC
Thai-HP-1-CD163     GAGACACGTG GGGCACCGTC TGTGATTCTG ACTTCTCTCT GGAGGCGGCC
Thai-EU-1-CD163     GAGACACGTG GGGCACCGTC TGTGATTCTG ACTTCTCTCT GGAGGCGGCC
Thai-US-1-CD163     GAGACACGTG GGGCACCGTC TGTGATTCTG ACTTCTCTCT GGAGGCGGCC

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                            1660       1670       1680       1690       1700
refCD163            AGCGTGCTGT GCAGGGAACT ACAGTGCGGC ACTGTGGTTT CCCTCCTGGG
Thai-HP-1-CD163     AGCGTGCTGT GCAGGGAACT ACAGTGCGGC ACTGTGGTTT CCCTCCTGGG
Thai-EU-1-CD163     AGCGTGCTGT GCAGGGAACT ACAGTGCGGC ACTGTGGTTT CCCTCCTGGG
Thai-US-1-CD163     AGCGTGCTGT GCAGGGAACT ACAGTGCGGC ACTGTGGTTT CCCTCCTGGG

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                            1710       1720       1730       1740       1750
refCD163            GGGAGCTCAC TTTGGAGAAG GAAGTGGACA GATCTGGGCT GAAGAATTCC
Thai-HP-1-CD163     GGGAGCTCAC TTTGGAGAAG GAAGTGGACA GATCTGGGCT GAAGAATTCC
Thai-EU-1-CD163     GGGAGCTCAC TTTGGAGAAG GAAGTGGACA GATCTGGGCT GAAGAATTCC
Thai-US-1-CD163     GGGAGCTCAC TTTGGAGAAG GAAGTGGACA GATCTGGGCT GAAGAATTCC

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                            1760       1770       1780       1790       1800
refCD163            AGTGTGAGGG GCACGAGTCC CACCTTTCAC TCTGCCCAGT AGCACCCCGC
Thai-HP-1-CD163     AGTGTGAGGG GCACGAGTCC CACCTTTCAC TCTGCCCAGT AGCACCCCGC
Thai-EU-1-CD163     AGTGTGAGGG GCACGAGTCC CACCTTTCAC TCTGCCCAGT AGCACCCCGC
Thai-US-1-CD163     AGTGTGAGGG GCACGAGTCC CACCTTTCAC TCTGCCCAGT AGCACCCCGC

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                            1810       1820       1830       1840       1850
refCD163            CCTGACGGGA CATGTAGCCA CAGCAGGGAC GTCGGCGTAG TCTGCTCAAG
Thai-HP-1-CD163     CCTGACGGGA CATGTAGCCA CAGCAGGGAC GTCGGCGTAG TCTGCTCAAG
Thai-EU-1-CD163     CCTGACGGGA CATGTAGCCA CAGCAGGGAC GTCGGCGTAG TCTGCTCAAG
Thai-US-1-CD163     CCTGACGGGA CATGTAGCCA CAGCAGGGAC GTCGGCGTAG TCTGCTCAAG

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                            1860       1870       1880       1890       1900
refCD163            ATACACACAA ATCCGCTTGG TGAATGGCAA GACCCCATGT GAAGGAAGAG
Thai-HP-1-CD163     ATACACACAA ATCCGCTTGG TGAATGGCAA GACCCCATGT GAAGGAAGAG
Thai-EU-1-CD163     ATACACACAA ATCCGCTTGG TGAATGGCAA GACCCCATGT GAAGGAAGAG
Thai-US-1-CD163     ATACACACAA ATCCGCTTGG TGAATGGCAA GACCCCATGT GAAGGAAGAG

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                            1910       1920       1930       1940       1950
refCD163            TGGAGCTCAA CATTCTTGGG TCCTGGGGGT CCCTCTGCAA CTCTCACTGG
Thai-HP-1-CD163     TGGAGCTCAA CATTCTTGGG TCCTGGGGGT CCCTCTGCAA CTCTCACTGG
Thai-EU-1-CD163     TGGAGCTCAA CATTCTTGGG TCCTGGGGGT CCCTCTGCAA CTCTCACTGG
Thai-US-1-CD163     TGGAGCTCAA CATTCTTGGG TCCTGGGGGT CCCTCTGCAA CTCTCACTGG

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                            1960       1970       1980       1990       2000
refCD163            GACATGGAAG ATGCCCATGT TTTATGCCAG CAGCTTAAAT GTGGAGTTGC
Thai-HP-1-CD163     GACATGGAAG ATGCCCATGT TTTATGCCAG CAGCTTAAAT GTGGAGTTGC
Thai-EU-1-CD163     GACATGGAAG ATGCCCATGT TTTATGCCAG CAGCTTAAAT GTGGAGTTGC
Thai-US-1-CD163     GACATGGAAG ATGCCCATGT TTTATGCCAG CAGCTTAAAT GTGGAGTTGC
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                            2010       2020       2030       2040       2050
refCD163            CCTTTCTATC CCGGGAGGAG CACCTTTTGG GAAAGGAAGT GAGCAGGTCT
Thai-HP-1-CD163     CCTTTCTATC CCGGGAGGAG CACCTTTTGG GAAAGGAAGT GAGCAGGTCT
Thai-EU-1-CD163     CCTTTCTATC CCGGGAGGAG CACCTTTTGG GAAAGGAAGT GAGCAGGTCT
Thai-US-1-CD163     CCTTTCTATC CCGGGAGGAG CACCTTTTGG GAAAGGAAGT GAGCAGGTCT

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                            2060       2070       2080       2090       2100
refCD163            GGAGGCACAT GTTTCACTGC ACTGGGACTG AGAAGCACAT GGGAGATTGT
Thai-HP-1-CD163     GGAGGCACAT GTTTCACTGC ACTGGGACTG AGAAGCACAT GGGAGACTGT
Thai-EU-1-CD163     GGAGGCACAT GTTTCACTGC ACTGGGACTG AGAAGCACAT GGGAGAGTGT
Thai-US-1-CD163     GGAGGCACAT GTTTCACTGC ACTGGGACTG AGAAGCACAT GGGAGACTGT

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                            2110       2120       2130       2140       2150
refCD163            TCCGTCACTG CTCTGGGCGC ATCACTCTGT TCTTCAGGGC AAGTGGCCTC
Thai-HP-1-CD163     TCCGTCACTG CTCTGGGCGC ATCACTCTGT TCTTCAGGGC AAGTGGCCTC
Thai-EU-1-CD163     TCCGTCACTG CTCTGGGCGC ATCACTCTGT TCTTCAGGGC AAGTGGCCTC
Thai-US-1-CD163     TCCGTCACTG CTCTGGGCGC ATCACTCTGT TCTTCAGGGC AAGTGGCCTC

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                            2160       2170       2180       2190       2200
refCD163            TGTAATCTGC TCAGGGAACC AGAGTCAGAC ACTATCCCCG TGCAATTCAT
Thai-HP-1-CD163     TGTAATCTGC TCAGGGAACC AGAGTCAGAC ACTATCCCCG TGCAATTCAT
Thai-EU-1-CD163     TGTAATCTGC TCAGGGAACC AGAGTCAGAC ACTATCCCCG TGCAATTCAT
Thai-US-1-CD163     TGTAATCTGC TCAGGGAACC AGAGTCAGAC ACTATCCCCG TGCAATTCAT

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                            2210       2220       2230       2240       2250
refCD163            CATCCTCGGA CCCATCAAGC TCTATTATTT CAGAAGAAAG TGGTGTTGCC
Thai-HP-1-CD163     CATCCTCGGA CCCATCAAGC TCTATTATTT CAGAAGAAAG TGGTGTTGCC
Thai-EU-1-CD163     CATCCTCGGA CCCATCAAGC TCTATTATTT CAGAAGAAAG TGGTGTTGCC
Thai-US-1-CD163     CATCCTCGGA CCCATCAAGC TCTATTATTT CAGAAGAAAG TGGTGTTGCC

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                            2260       2270       2280       2290       2300
refCD163            TGCATAGGGA GTGGTCAACT TCGCCTGGTC GATGGAGGTG GTCGTTGTGC
Thai-HP-1-CD163     TGCATAGGGA GTGGTCAACT TCGCCTGGTC GATGGAGGTG GTCGTTGTGC
Thai-EU-1-CD163     TGCATAGGGA GTGGTCAACT TCGCCTGGTC GATGGAGGTG GTCGTTGTGC
Thai-US-1-CD163     TGCATAGGGA GTGGTCAACT TCGCCTGGTC GATGGAGGTG GTCGTTGTGC

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                            2310       2320       2330       2340       2350
refCD163            TGGGAGAGTA GAGGTCTATC CTGGGGCATC CTGGGGCACC ATCTGTGATG
Thai-HP-1-CD163     TGGGAGAGTA GAGGTCTATC CTGGGGCATC CTGGGGCACC ATCTGTGATG
Thai-EU-1-CD163     TGGGAGAGTA GAGGTCTATC CTGGGGCATC CTGGGGCACC ATCTGTGATG
Thai-US-1-CD163     TGGGAGAGTA GAGGTCTATC CTGGGGCATC CTGGGGCACC ATCTGTGATG

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                            2360       2370       2380       2390       2400
refCD163            ACAGCTGGGA CCTGAATGAT GCCCATGTGG TGTGCAAACA GCTGAGCTGT
Thai-HP-1-CD163     ACAGCTGGGA CCTGAATGAT GCCCATGTGG TGTGCAAACA GCTGAGCTGT
Thai-EU-1-CD163     ACAGCTGGGA CCTGAATGAT GCCCATGTGG TGTGCAAACA GCTGAGCTGT
Thai-US-1-CD163     ACAGCTGGGA CCTGAATGAT GCCCATGTGG TGTGCAAACA GCTGAGCTGT
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                            2410       2420       2430       2440       2450
refCD163            GGATGGGCCA TTAATGCCAC TGGTTCTGCT CATTTTGGGG AAGGAACAGG
Thai-HP-1-CD163     GGATGGGCCA TTAATGCCAC TGGTTCTGCT CATTTTGGGG AAGGAACAGG
Thai-EU-1-CD163     GGATGGGCCA TTAATGCCAC TGGTTCTGCT CATTTTGGGG AAGGAACAGG
Thai-US-1-CD163     GGATGGGCCA TTAATGCCAC TGGTTCTGCT CATTTTGGGG AAGGAACAGG

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                            2460       2470       2480       2490       2500
refCD163            GCCCATTTGG CTGGATGAGA TAAACTGTAA TGGAAAAGAA TCTCATATTT
Thai-HP-1-CD163     GCCCATTTGG CTGGATGAGA TAAACTGTAA TGGAAAAGAA TCTCATATTT
Thai-EU-1-CD163     GCCCATTTGG CTGGATGAGA TAAACTGTAA TGGAAAAGAA TCTCATATTT
Thai-US-1-CD163     GCCCATTTGG CTGGATGAGA TAAACTGTAA TGGAAAAGAA TCTCATATTT

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                            2510       2520       2530       2540       2550
refCD163            GGCAATGCCA CTCACATGGT TGGGGGCGGC ACAATTGCAG GCATAAGGAG
Thai-HP-1-CD163     GGCAATGCCA CTCACATGGT TGGGGGCGGC ACAATTGCAG GCATAAGGAG
Thai-EU-1-CD163     GGCAATGCCA CTCACATGGT TGGGGGCGGC ACAATTGCAG GCATAAGGAG
Thai-US-1-CD163     GGCAATGCCA CTCACATGGT TGGGGGCGGC ACAATTGCAG GCATAAGGAG

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                            2560       2570       2580       2590       2600
refCD163            GATGCAGGAG TCATCTGCTC AGAGTTCATG TCTCTGAGAC TGATCAGTGA
Thai-HP-1-CD163     GATGCAGGAG TCATCTGCTC AGAGTTCATG TCTCTGGGAC TGATCAGTGA
Thai-EU-1-CD163     GATGCAGGAG TCATCTGCCC AGAGTTCATG TCTCTGGGAC TGATCAGTGA
Thai-US-1-CD163     GATGCAGGAG TCATCTGCTC AGAGTTCATG TCTCTGAGAC TGATCAGTGA

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                            2610       2620       2630       2640       2650
refCD163            AAACAGCAGA GAGACCTGTG CAGGGCGCCT GGAAGTTTTT TACAACGGAG
Thai-HP-1-CD163     AAACAGCAGA GAGACCTGTG CAGGGCGCCT GGAAGTTTTT TACAACGGAG
Thai-EU-1-CD163     AAACAGCAGA GAGACCTGTG CAGGGCGCCT GGAAGTTTTT TACAACGGAG
Thai-US-1-CD163     AAACAGCAGA GAGACCTGTG CAGGGCGCCT GGAAGTTTTT TACAACGGAG

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                            2660       2670       2680       2690       2700
refCD163            CTTGGGGCAG CGTTGGCAGG AATAGCATGT CTCCAGCCAC AGTGGGGGTG
Thai-HP-1-CD163     CTTGGGGCAG CGTTGGCAGG AATAGCATGT CTCCAGCCAC AGTGGGGGTG
Thai-EU-1-CD163     CTTGGGGCAG CGTTGGCAGG AATAGCATGT CTCCAGCCAC AGTGGGGGTG
Thai-US-1-CD163     CTTGGGGCAG CGTTGGCAGG AATAGCATGT CTCCAGCCAC AGTGGGGGTG

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                            2710       2720       2730       2740       2750
refCD163            GTATGCAGGC AGCTGGGCTG TGCAGACAGA GGGGACATCA GCCCTGCATC
Thai-HP-1-CD163     GTATGCAGGC AGCTGGGCTG TGCAGACAGA GGGGACATCA GCCCTGCATC
Thai-EU-1-CD163     GTATGCAGGC AGCTGGGCTG TGCAGACAGA GGGGACATCA GCCCTGCATC
Thai-US-1-CD163     GTATGCAGGC AGCTGGGCTG TGCAGACAGA GGGGACATCA GCCCTGCATC

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                            2760       2770       2780       2790       2800
refCD163            TTCAGACAAG ACAGTGTCCA GGCACATGTG GGTGGACAAT GTTCAGTGTC
Thai-HP-1-CD163     TTCAGACAAG ACAGTGTCCA GGCACATGTG GGTGGACAAT GTTCAGTGTC
Thai-EU-1-CD163     TTCAGACAAG ACAGTGTCCA GGCACATGTG GGTGGACAAT GTTCAGTGTC
Thai-US-1-CD163     TTCAGACAAG ACAGTGTCCA GGCACATGTG GGTGGACAAT GTTCAGTGTC
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                           2810       2820       2830       2840       2850
refCD163            CTAAAGGACC TGACACACTA TGGCAGTGCC CCTCATCTCC ATGGAAGAAG
Thai-HP-1-CD163     CTAAAGGACC TGACACACTA TGGCAGTGCC CATCATCTCC ATGGAAGAAG
Thai-EU-1-CD163     CTAAAGGACC TGACACACTA TGGCAGTGCC CATCATCTCC ATGGAAGAAG
Thai-US-1-CD163     CTAAAGGACC TGACACACTA TGGCAGTGCC CATCATCTCC ATGGAAGAAG

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                           2860       2870       2880       2890       2900
refCD163            AGACTGGCCA GCCCCTCAGA GGAGACATGG ATCACATGTG CCAACAAAAT
Thai-HP-1-CD163     AGACTGGCCA GCCCCTCAGA GGAGACATGG ATCACATGTG CCAACAAAAT
Thai-EU-1-CD163     AGACTGGCCA GCCCCTCAGA GGAGACATGG ATCACATGTG CCAACAAAAT
Thai-US-1-CD163     AGACTGGCCA GCCCCTCAGA GGAGACATGG ATCACATGTG CCAACAAAAT

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                           2910       2920       2930       2940       2950
refCD163            AAGACTTCAA GAAGGAAACA CTAATTGTTC TGGACGTGTG GAGATCTGGT
Thai-HP-1-CD163     AAGACTTCAA GAAGGAAACA CTAATTGTTT TGGACGTGTG GAGATCTGGT
Thai-EU-1-CD163     AAGACTTCAA GAAGGAAACA CTAATTGTTC TGGACGTGTG GAGATCTGGT
Thai-US-1-CD163     AAGACTTCAA GAAGGAAACA CTAATTGTTC TGGACGTGTG GAGATCTGGT

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                           2960       2970       2980       2990       3000
refCD163            ACGGAGGTTC CTGGGGCACT GTGTGTGACG ACTCCTGGGA CCTTGAAGAT
Thai-HP-1-CD163     ACGGAGGTTC CTGGGGCACT GTGTGTGACG ACTCCTGGGA CCTTGAAGAT
Thai-EU-1-CD163     ACGGAGGTTC CTGGGGCACT GTGTGTGACG ACTCCTGGGA CCTTGAAGAT
Thai-US-1-CD163     ACGGAGGTTC CTGGGGCACT GTGTGTGACG ACTCCTGGGA CCTTGAAGAT

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                           3010       3020       3030       3040       3050
refCD163            GCTCAGGTGG TGTGCCGACA GCTGGGCTGT GGCTCAGCTT TGGAGGCAGG
Thai-HP-1-CD163     GCTCAGGTGG TGTGCCGACA GCTGGGCTAA GGCTCAGCTT TGGAGGCAGG
Thai-EU-1-CD163     GCTCAGGTGG TGTGCCGACA GCTGGGCTGT GGCTCAGCTT TGGAGGCAGG
Thai-US-1-CD163     GCTCAGGTGG TGTGCCGACA GCTGGGCTGT GGCTCAGCTT TGGAGGCAGG

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                           3060       3070       3080       3090       3100
refCD163            AAAAGAGCCC GCATTTGGCC AGGGGACTGG GCCCATATGG CTCAATGAAG
Thai-HP-1-CD163     AAAAGAGCCC GCATTTGGCC AGGGGACTGG GCCCATATGG CTCAATGAAG
Thai-EU-1-CD163     AAAAGAGCCC GCATTTGGCC AGGGGACTGG GCCCATATGG CTCAATGAAG
Thai-US-1-CD163     AAAAGAGCCC GCATTTGGCC AGGGGACTGG GCCCATATGG CTCAATGAAG

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                           3110       3120       3130       3140       3150
refCD163            TGAAGTGCAA GGGGAATGAA CCCTCCTTGT GGGATTGTCC TGCCAGATCC
Thai-HP-1-CD163     TGAAGTGCAA GGGGAATGAA CCCTCCTTGT GGGATTGTCC TGCCAGATCC
Thai-EU-1-CD163     TGAAGTGCAA GGGGAATGAA CCCTCCTTGT GGGATTGTCC TGCCAGATCC
Thai-US-1-CD163     TGAAGTGCAA GGGGAATGAA CCCTCCTTGT GGGATTGTCC TGCCAGATCC

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                           3160       3170       3180       3190       3200
refCD163            TGGGGCCACA GTGACTGTGG ACACAAGGAG GATGCTGCTG TGACGTGCTC
Thai-HP-1-CD163     TGGGGCCACA GTGACTGTGG ACACAAGGAG GATGCTGCTG TGACGTGCTC
Thai-EU-1-CD163     TGGGGCCACA GTGACTGTGG ACACAAGGAG GATGCTGCTG TGACGTGCTC
Thai-US-1-CD163     TGGGGCCACA GTGACTGTGG ACACAAGGAG GATGCTGCTG TGACGTGCTC
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                            3210       3220       3230       3240       3250
refCD163            AGAAATTGCA AAGAGCCGAG AATCCCTACA TGCCACAGGT CGCTCATCTT
Thai-HP-1-CD163     AGAAATTGCA AAGAGCCGAG AATCCCTACA TGCCACAGGT CGCTCATCTT
Thai-EU-1-CD163     AGAAATTGCA AAGAGCCGAG AATCCCTACA TGCCACAGGT CGCTCATCTT
Thai-US-1-CD163     AGAAATTGCA AAGAGCCGAG AATCCCTACA TGCCACAGGT CGCTCATCTT

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                            3260       3270       3280       3290       3300
refCD163            TTGTTGCACT TGCAATCTTT GGGGTCATTC TGTTGGCCTG TCTCATCGCA
Thai-HP-1-CD163     TTGTTGCACT TGCAATCTTT GGGGTCATTC TGTTGGCCTG TCTCATCGCA
Thai-EU-1-CD163     TTGTTGCACT TGCAATCTTT GGGGTCATTC TGTTGGCCTG TCTCATCGCA
Thai-US-1-CD163     TTGTTGCACT TGCAATCTTT GGGGTCATTC TGTTGGCCTG TCTCATCGCA

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                            3310       3320       3330       3340       3350
refCD163            TTCCTCATTT GGACTCAGAA GCGAAGACAG AGGCAGCGGC TCTCAGTTTT
Thai-HP-1-CD163     TTCCTCATTT GGACTCAGAA GCGAAGACAG AGGCAGCGGC TCTCAGTTTT
Thai-EU-1-CD163     TTCCTCATTT GGACTCAGAA GCGAAGACAG AGGCAGCGGC TCTCAGTTTT
Thai-US-1-CD163     TTCCTCATTT GGACTCAGAA GCGAAGACAG AGGCAGCGGC TCTCAGTTTT

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                            3360       3370       3380       3390       3400
refCD163            CTCAGGAGGA GAGAATTCTG TCCATCAAAT TCAATACCGG GAGATGAATT
Thai-HP-1-CD163     CTCAGGAGGA GAGAATTCTG TACATCAAAT TCAATACCGG GAGATGAATT
Thai-EU-1-CD163     CTCAGGAGGA GAGAATTCTG TACATCAAAT TCAATACCGG GAGATGAATT
Thai-US-1-CD163     CTCAGGAGGA GAGAATTCTG TACATCAAAT TCAATACCGG GAGATGAATT

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                            3410       3420       3430       3440       3450
refCD163            CTTGCCTGAA AGCAGATGAA ACGGATATGC TAAATCCCTC AGGAGACCAC
Thai-HP-1-CD163     CTTGCCTGAA AGCAGATGAA ACGGATATGC TAAATCCCTC AGGAGACCAC
Thai-EU-1-CD163     CTTGCCTGAA AGCAGATGAA ACGGATATGC TAAATCCCTC AGGAGACCAC
Thai-US-1-CD163     CTTGCCTGAA AGCAGATGAA ACGGATATGC TAAATCCCTC AGGAGACCAC

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                            3460       3470       3480       3490       3500
refCD163            TCTGAAGTAC AATGAAAAGG AAAATGGGAA TTATAACCTG GTGAGTTCAG
Thai-HP-1-CD163     TCTGAAGTAC AATGAAAAGG AAAATGGGAA TTATAACCTG GTGAGTTCAG
Thai-EU-1-CD163     TCTGAAGTAC AATGAAAAGG AAAATGGGAA TTATAACCTG GTGAGTTCAG
Thai-US-1-CD163     TCTGAAGTAC AATGAAAAGG AAAATGGGAA TTATAACCTG GTGAGTTCAG

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                             3510       3520       3530
Sus scrofa           CCTTTAAGAT ACCTTGATGA AGACCTGGAC TA
Thai-HP-1-CD163      CCTTTAAGAT ACCTTGATGA AGACCTGGAC TA
Thai-EU-1-CD163      CCTTTAAGAT ACCTTGATGA AGACCTGGAC TA
Thai-US-1-CD163      CCTTTAAGAT ACCTTGATGA AGACCTGGAC TA
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Figure 4.13 Nucleotide sequence alignments of the reference CD163 (refCD163) (accession number is NM_213976) with recombinant plasmids of Thai-HP-1-pCR®-XL-TOPO®-CD163 (Thai-HP-1-CD163), Thai-EU-1-pCR®-XL-TOPO®-CD163 (Thai-EU-1-CD163) and Thai-US-1-pCR®-XL-TOPO®-CD163 (Thai-US-1-CD163), respectively. The nucleotide changes are boxed.

Table 4.8 Deduced amino acid sequences of 3 recombinant porcine CD163 (full) plasmids

Product's name	Sequence
1. Thai-HP-1-CD163 (1,133 aa)	MVLLEDSGSADFRRCSAHLSSFTFAVVAVLSACLVTSSLGGKDKELRLTGGE NKCSGRVEVKVQEEWGTVCNNGWDMDVVSVVCRQLGCPTAIKATGWANFS AGSGRIWMDHVSCRGNESALWDCKHDGWGKHNCTHQQDAGVTCSDGSD LEMRLVNGGNRCLGRIEVKFQERWGTVCDDNFNINHASVVCKQLECGSAVS FSGSANFGEGSGPIWFDDLVCNGNESALWNCKHEGWGKHNCDHAEDAGV ICLNGADLKLRVVDGVTECSGRLEVKFQGEWGTICDDGWDSDDAAVACKQ LGCPTAVTAIGRVNASEGTGHIWLDSVSCHGHESALWQCRHHEWGKHYCN HNEDAGVTCSDGSDLELRLKGGGSHCAGTVEVEIQKLVGKVCDRSWGLKE ADVVCRQLGCGSALKTSYQVYSKTKATNTWLFVSSCNGNETSLWDCKNWQ WGGLSCAHYDEAKITCSAHRKPRLVGGDIPCSGRVEVQHGDTWGTVCDSD FSLEAASVLCRELQCGTVVSLLGGAHFGEGSGQIWAEEFQCEGHESHLSLC PVAPRPDGTCSHSRDVGVVCSRYTQIRLVNGKTPCEGRVELNILGSWGSLCN SHWDMEDAHVLCQQLKCGVALSIPGGAPFGKGSEQVWRHMFHCTGTEKH MGDCSVTALGASLCSSGQVASVICSGNQSQTLSPCNSSSSDPSSSIISEESGV ACIGSGQLRLVDGGGRCAGRVEVYPGASWGTICDDSWDLNDAHVVCKQLS CGWAINATGSAHFGEGTGPIWLDEINCNGKESHIWQCHSHGWGRHNCRHK EDAGVICSEFMSLGLISENSRETCAGRLEVFYNGAWGSVGRNSMSPATVGVV CRQLGCADRGDISPASSDKTVSRHMWVDNVQCPKGPDTLWQCPSSPWKK RLASPSEETWITCANKIRLQEGNTNCFGRVEIWYGGSWGTVCDDSWDLEDA QVVCRQLGGSALEAGKEPAFGQGTGPIWLNEVKCKGNEPSLWDCPARSWG HSDCGHKEDAAVTCSEIAKSRESLHATGRSSFVALAIFGVILLACLIAFLIWTQ KRRQRQRLSVFSGGENSVHQIQYREMNSCLKADETDMLNPSGDHSEVQ*K GKWEL*PGEFSL*DTLMKTWT
2. Thai-EU-1-	MVLLEDSGSADFRRCSAHLSSFTFAVVAVLSACLVTSSLGGKDKELRLTGGE

CD163 (1,133 aa)	NKCSGRVEVKVQEEWGTVCNNGWDMDVVSVVCRQLGCPTAIKATGWANFS AGSGRIWMDHVSCRGNESALWDCKHDGWGKHNCTHQQDAGVTCSDGSD LEMRLVNGGNRCLGRIEVKFQERWGTVCDDNFNINHASVVCKQLECGSAVS FSGSANFGEGSGPIWFDDLVCNGNESALWNCKHEGWGKHNCDHAEDAGV ICLNGADLKLRVVDGVTECSGRLEVKFQGEWGTICDDGWDSDDAAVACKQ LGCPTAVTAIGRVNASEGTGHIWLDSVSCHGHESALWQCRHHEWGKHYCN HNEDAGVTCSDGSDLELRLKGGGSHCAGTVEVEIQKLVGKVCDRSWGLKE ADVVCRQLGCGSALKTSYQVYSKTKATNTWLFVSSCNGNETSLWDCKNWQ WGGLSCDHYDEAKITCSAHRKPRLVGGDIPCSGRVEVQHGDTWGTVCDSD FSLEAASVLCRELQCGTVVSLLGGAHFGEGSGQIWAEEFQCEGHESHLSLC PVAPRPDGTCSHSRDVGVVCSRYTQIRLVNGKTPCEGRVELNILGSWGSLCN SHWDMEDAHVLCQQLKCGVALSIPGGAPFGKGSEQVWRHMFHCTGTEKH MGECSVTALGASLCSSGQVASVICSGNQSQTLSPCNSSSSDPSSSIISEESGV ACIGSGQLRLVDGGGRCAGRVEVYPGASWGTICDDSWDLNDAHVVCKQLS CGWAINATGSAHFGEGTGPIWLDEINCNGKESHIWQCHSHGWGRHNCRHK EDAGVICPEFMSLGLISENSRETCAGRLEVFYNGAWGSVGRNSMSPATVGVV CRQLGCADRGDISPASSDKTVSRHMWVDNVQCPKGPDTLWQCPSSPWKK RLASPSEETWITCANKIRLQEGNTNCSGRVEIWYGGSWGTVCDDSWDLEDA QVVCRQLGCGSALEAGKEPAFGQGTGPIWLNEVKCKGNEPSLWDCPARSW GHSDCGHKEDAAVTCSEIAKSRESLHATGRSSFVALAIFGVILLACLIAFLIWT QKRRQRQRLSVFSGGENSVHQIQYREMNSCLKADETDMLNPSGDHSEVQ* KGKWEL*PGEFSL*DTLMKTWT
3. Thai-US-1-CD163 (1,133 aa)	MVLLEDSGSADFRRCSAHLSSFTFAVVAVLSACLVTSSLGGKDKELRLTGGE NKCSGRVEVKVQEEWGTVCNNGWDMDVVSVVCRQLGCPTAIKATGWANFS AGSGRIWMDHVSCRGNESALWDCKHDGWGKHNCTHQQDAGVTCSDGSD LEMRLVNGGNRCLGRIEVKFQERWGTVCDDNFNINHASVVCKQLECGSAVS FSGSANFGEGSGPIWFDDLVCNGNESALWNCKHEGWGKHNCDHAEDAGV

ICLNGADLKLRVVDGVTECSGRLEVKFQGEWGTICDDGWDSDDAAVACKQ
LGCPTAVTAIGRVNASEGTGHIWLDSVSCHGHESALWQCRHHEWGKHYCN
HNEDAGVTCSDGSDLELRLKGGGSHCAGTVEVEIQKLVGKVCDRSWGLKE
ADVVCRQLGCGSALKTSYQVYSKTKATNTWLFVSSCNGNETSLWDCKNWQ
WGGLSCAHYDEAKITCSAHRKPRLVGGDIPCSGRVEVQHGDTWGTVCDSD
FSLEAASVLCRELQCGTVVSLLGGAHFGEGSGQIWAEEFQCEGHESHLSLC
PVAPRPDGTCSHSRDVGVVCSRYTQIRLVNGKTPCEGRVELNILGSWGSLCN
SHWDMEDAHVLCQQLKCGVALSIPGGAPFGKGSEQVWRHMFHCTGTEKH
MGDCSVTALGASLCSSGQVASVICSGNQSQTLSPCNSSSSDPSSSIISEESGV
ACIGSGQLRLVDGGGRCAGRVEVYPGASWGTICDDSWDLNDAHVVCKQLS
CGWAINATGSAHFGEGTGPIWLDEINCNGKESHIWQCHSHGWGRHNCRHK
EDAGVICSEFMSLRLISENSRETCAGRLEVFYNGAWGSVGRNSMSPATVGVV
CRQLGCADRGDISPASSDKTVSRHMWVDNVQCPKGPDTLWQCPSSPWKK
RLASPSEETWITCANKIRLQEGNTNCSGRVEIWYGGSWGTVCDDSWDLEDA
QVVCRQLGCGSALEAGKEPAFGQGTGPIWLNEVKCKGNEPSLWDCPARSW
GHSDCGHKEDAAVTCSEIAKSRESLHATGRSSFVALAIFGVILLACLIAFLIWT
QKRRQRQRLSVFSGGENSVHQIQYREMNSCLKADETDMLNPSGDHSEVQ*
KGKWEL*PGEFSL*DTLMKTWT

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                             10         20         30         40         50
refCD163            MVLLEDSGSA DFRRCSAHLS SFTFAVVAVL SACLVTSSLG GKDKELRLTG
Thai-HP-1-CD163     MVLLEDSGSA DFRRCSAHLS SFTFAVVAVL SACLVTSSLG GKDKELRLTG
Thai-EU-1-CD163     MVLLEDSGSA DFRRCSAHLS SFTFAVVAVL SACLVTSSLG GKDKELRLTG
Thai-US-1-CD163     MVLLEDSGSA DFRRCSAHLS SFTFAVVAVL SACLVTSSLG GKDKELRLTG

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                             60         70         80         90        100
refCD163            GENKCSGRVE VKVQEEWGTV CNNGWDMDVV SVVCRQLGCP TAIKATGWAN
Thai-HP-1-CD163     GENKCSGRVE VKVQEEWGTV CNNGWDMDVV SVVCRQLGCP TAIKATGWAN
Thai-EU-1-CD163     GENKCSGRVE VKVQEEWGTV CNNGWDMDVV SVVCRQLGCP TAIKATGWAN
Thai-US-1-CD163     GENKCSGRVE VKVQEEWGTV CNNGWDMDVV SVVCRQLGCP TAIKATGWAN

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                            110        120        130        140        150
refCD163            FSAGSGRIWM DHVSCRGNES ALWDCKHDGW GKHNCTHQQD AGVTCSDGSD
Thai-HP-1-CD163     FSAGSGRIWM DHVSCRGNES ALWDCKHDGW GKHNCTHQQD AGVTCSDGSD
Thai-EU-1-CD163     FSAGSGRIWM DHVSCRGNES ALWDCKHDGW GKHNCTHQQD AGVTCSDGSD
Thai-US-1-CD163     FSAGSGRIWM DHVSCRGNES ALWDCKHDGW GKHNCTHQQD AGVTCSDGSD

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                            160        170        180        190        200
refCD163            LEMRLVNGGN RCLGRIEVKF QERWGTVCDD NFNINHASVV CKQLECGSAV
Thai-HP-1-CD163     LEMRLVNGGN RCLGRIEVKF QERWGTVCDD NFNINHASVV CKQLECGSAV
Thai-EU-1-CD163     LEMRLVNGGN RCLGRIEVKF QERWGTVCDD NFNINHASVV CKQLECGSAV
Thai-US-1-CD163     LEMRLVNGGN RCLGRIEVKF QERWGTVCDD NFNINHASVV CKQLECGSAV

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                            210        220        230        240        250
refCD163            SFSGSANFGE GSGPIWFDDL VCNGNESALW NCKHEGWGKH NCDHAEDAGV
Thai-HP-1-CD163     SFSGSANFGE GSGPIWFDDL VCNGNESALW NCKHEGWGKH NCDHAEDAGV
Thai-EU-1-CD163     SFSGSANFGE GSGPIWFDDL VCNGNESALW NCKHEGWGKH NCDHAEDAGV
Thai-US-1-CD163     SFSGSANFGE GSGPIWFDDL VCNGNESALW NCKHEGWGKH NCDHAEDAGV

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                            260        270        280        290        300
refCD163            ICLNGADLKL RVVDGLTECS GRLEVKFQGE WGTICDDGWD SDDAAVACKQ
Thai-HP-1-CD163     ICLNGADLKL RVVDGVTECS GRLEVKFQGE WGTICDDGWD SDDAAVACKQ
Thai-EU-1-CD163     ICLNGADLKL RVVDGVTECS GRLEVKFQGE WGTICDDGWD SDDAAVACKQ
Thai-US-1-CD163     ICLNGADLKL RVVDGVTECS GRLEVKFQGE WGTICDDGWD SDDAAVACKQ

                    ....|....| ....|....| ....|....| ....|....| ....|....|
                            310        320        330        340        350
refCD163            LGCPTAVTAI GRVNASEGTG HIWLDSVSCH GHESALWQCR HHEWGKHYCN
Thai-HP-1-CD163     LGCPTAVTAI GRVNASEGTG HIWLDSVSCH GHESALWQCR HHEWGKHYCN
Thai-EU-1-CD163     LGCPTAVTAI GRVNASEGTG HIWLDSVSCH GHESALWQCR HHEWGKHYCN
Thai-US-1-CD163     LGCPTAVTAI GRVNASEGTG HIWLDSVSCH GHESALWQCR HHEWGKHYCN

                   ....|....| ....|....| ....|....| ....|....| ....|....|
                           360        370        380        390        400
refCD163            HNEDAGVTCS DGSDLELRLK GGGSHCAGTV EVEIQKLVGK VCDRSWGLKE
Thai-HP-1-CD163     HNEDAGVTCS DGSDLELRLK GGGSHCAGTV EVEIQKLVGK VCDRSWGLKE
Thai-EU-1-CD163     HNEDAGVTCS DGSDLELRLK GGGSHCAGTV EVEIQKLVGK VCDRSWGLKE
Thai-US-1-CD163     HNEDAGVTCS DGSDLELRLK GGGSHCAGTV EVEIQKLVGK VCDRSWGLKE
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                    ....|....| ....|....| ....|....| ....|....| ....|....|
                            410        420        430        440        450
refCD163            ADVVCRQLGC GSALKTSYQV YSKTKATNTW LFVSSCNGNE TSLWDCKNWQ
Thai-HP-1-CD163     ADVVCRQLGC GSALKTSYQV YSKTKATNTW LFVSSCNGNE TSLWDCKNWQ
Thai-EU-1-CD163     ADVVCRQLGC GSALKTSYQV YSKTKATNTW LFVSSCNGNE TSLWDCKNWQ
Thai-US-1-CD163     ADVVCRQLGC GSALKTSYQV YSKTKATNTW LFVSSCNGNE TSLWDCKNWQ

                    ....|....| ....|....| ....|....| ....|....| ....|....|
                            460        470        480        490        500
refCD163            WGGLSGDHYD EAKITCSAHR KPRLVGGDIP CSGRVEVQHG DTWGTVCDSD
Thai-HP-1-CD163     WGGLSGAHYD EAKITCSAHR KPRLVGGDIP CSGRVEVQHG DTWGTVCDSD
Thai-EU-1-CD163     WGGLSGDHYD EAKITCSAHR KPRLVGGDIP CSGRVEVQHG DTWGTVCDSD
Thai-US-1-CD163     WGGLSGAHYD EAKITCSAHR KPRLVGGDIP CSGRVEVQHG DTWGTVCDSD

                    ....|....| ....|....| ....|....| ....|....| ....|....|
                            510        520        530        540        550
refCD163            FSLEAASVLC RELQCGTVVS LLGGAHFGEG SGQIWAEEFQ CEGHESHLSL
Thai-HP-1-CD163     FSLEAASVLC RELQCGTVVS LLGGAHFGEG SGQIWAEEFQ CEGHESHLSL
Thai-EU-1-CD163     FSLEAASVLC RELQCGTVVS LLGGAHFGEG SGQIWAEEFQ CEGHESHLSL
Thai-US-1-CD163     FSLEAASVLC RELQCGTVVS LLGGAHFGEG SGQIWAEEFQ CEGHESHLSL

                    ....|....| ....|....| ....|....| ....|....| ....|....|
                            560        570        580        590        600
refCD163            CPVAPRPDGT CSHSRDVGVV CSRYTQIRLV NGKTPCEGRV ELNILGSWGS
Thai-HP-1-CD163     CPVAPRPDGT CSHSRDVGVV CSRYTQIRLV NGKTPCEGRV ELNILGSWGS
Thai-EU-1-CD163     CPVAPRPDGT CSHSRDVGVV CSRYTQIRLV NGKTPCEGRV ELNILGSWGS
Thai-US-1-CD163     CPVAPRPDGT CSHSRDVGVV CSRYTQIRLV NGKTPCEGRV ELNILGSWGS

                    ....|....| ....|....| ....|....| ....|....| ....|....|
                            610        620        630        640        650
refCD163            LCNSHWDMED AHVLCQQLKC GVALSIPGGA PFGKGSEQVW RHMFHCTGTE
Thai-HP-1-CD163     LCNSHWDMED AHVLCQQLKC GVALSIPGGA PFGKGSEQVW RHMFHCTGTE
Thai-EU-1-CD163     LCNSHWDMED AHVLCQQLKC GVALSIPGGA PFGKGSEQVW RHMFHCTGTE
Thai-US-1-CD163     LCNSHWDMED AHVLCQQLKC GVALSIPGGA PFGKGSEQVW RHMFHCTGTE

                    ....|....| ....|....| ....|....| ....|....| ....|....|
                            660        670        680        690        700
refCD163            KHMGDCSVTA LGASLCSSGQ VASVICSGNQ SQTLSPCNSS SSDPSSSIIS
Thai-HP-1-CD163     KHMGDCSVTA LGASLCSSGQ VASVICSGNQ SQTLSPCNSS SSDPSSSIIS
Thai-EU-1-CD163     KHMGECSVTA LGASLCSSGQ VASVICSGNQ SQTLSPCNSS SSDPSSSIIS
Thai-US-1-CD163     KHMGDCSVTA LGASLCSSGQ VASVICSGNQ SQTLSPCNSS SSDPSSSIIS

                    ....|....| ....|....| ....|....| ....|....| ....|....|
                            710        720        730        740        750
refCD163            EESGVACIGS GQLRLVDGGG RCAGRVEVYP GASWGTICDD SWDLNDAHVV
Thai-HP-1-CD163     EESGVACIGS GQLRLVDGGG RCAGRVEVYP GASWGTICDD SWDLNDAHVV
Thai-EU-1-CD163     EESGVACIGS GQLRLVDGGG RCAGRVEVYP GASWGTICDD SWDLNDAHVV
Thai-US-1-CD163     EESGVACIGS GQLRLVDGGG RCAGRVEVYP GASWGTICDD SWDLNDAHVV

                    ....|....| ....|....| ....|....| ....|....| ....|....|
                            760        770        780        790        800
refCD163            CKQLSCGWAI NATGSAHFGE GTGPIWLDEI NCNGKESHIW QCHSHGWGRH
Thai-HP-1-CD163     CKQLSCGWAI NATGSAHFGE GTGPIWLDEI NCNGKESHIW QCHSHGWGRH
Thai-EU-1-CD163     CKQLSCGWAI NATGSAHFGE GTGPIWLDEI NCNGKESHIW QCHSHGWGRH
Thai-US-1-CD163     CKQLSCGWAI NATGSAHFGE GTGPIWLDEI NCNGKESHIW QCHSHGWGRH
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                    ....|....| ....|....| ....|....| ....|....| ....|....|
                            810        820        830        840        850
refCD163            NCRHKEDAGV ICSEFMSLRL ISENSRETCA GRLEVFYNGA WGSVGRNSMS
Thai-HP-1-CD163     NCRHKEDAGV ICSEFMSLGL ISENSRETCA GRLEVFYNGA WGSVGRNSMS
Thai-EU-1-CD163     NCRHKEDAGV ICPEFMSLGL ISENSRETCA GRLEVFYNGA WGSVGRNSMS
Thai-US-1-CD163     NCRHKEDAGV ICSEFMSLRL ISENSRETCA GRLEVFYNGA WGSVGRNSMS

                    ....|....| ....|....| ....|....| ....|....| ....|....|
                            860        870        880        890        900
refCD163            PATVGVVCRQ LGCADRGDIS PASSDKTVSR HMWVDNVQCP KGPDTLWQCP
Thai-HP-1-CD163     PATVGVVCRQ LGCADRGDIS PASSDKTVSR HMWVDNVQCP KGPDTLWQCP
Thai-EU-1-CD163     PATVGVVCRQ LGCADRGDIS PASSDKTVSR HMWVDNVQCP KGPDTLWQCP
Thai-US-1-CD163     PATVGVVCRQ LGCADRGDIS PASSDKTVSR HMWVDNVQCP KGPDTLWQCP

                    ....|....| ....|....| ....|....| ....|....| ....|....|
                            910        920        930        940        950
refCD163            SSPWKKRLAS PSEETWITCA NKIRLQEGNT NCSGRVEIWY GGSWGTVCDD
Thai-HP-1-CD163     SSPWKKRLAS PSEETWITCA NKIRLQEGNT NCFGRVEIWY GGSWGTVCDD
Thai-EU-1-CD163     SSPWKKRLAS PSEETWITCA NKIRLQEGNT NCSGRVEIWY GGSWGTVCDD
Thai-US-1-CD163     SSPWKKRLAS PSEETWITCA NKIRLQEGNT NCSGRVEIWY GGSWGTVCDD

                    ....|....| ....|....| ....|....| ....|....| ....|....|
                           960        970        980        990       1000
refCD163            SWDLEDAQVV CRQLGCGSAL EAGKEPAFGQ GTGPIWLNEV KCKGNEPSLW
Thai-HP-1-CD163     SWDLEDAQVV CRQLG*GSAL EAGKEPAFGQ GTGPIWLNEV KCKGNEPSLW
Thai-EU-1-CD163     SWDLEDAQVV CRQLGCGSAL EAGKEPAFGQ GTGPIWLNEV KCKGNEPSLW
Thai-US-1-CD163     SWDLEDAQVV CRQLGCGSAL EAGKEPAFGQ GTGPIWLNEV KCKGNEPSLW

                    ....|....| ....|....| ....|....| ....|....| ....|....|
                           1010       1020       1030       1040       1050
refCD163            DCPARSWGHS DCGHKEDAAV TCSEIAKSRE SLHATGRSSF VALAIFGVIL
Thai-HP-1-CD163     DCPARSWGHS DCGHKEDAAV TCSEIAKSRE SLHATGRSSF VALAIFGVIL
Thai-EU-1-CD163     DCPARSWGHS DCGHKEDAAV TCSEIAKSRE SLHATGRSSF VALAIFGVIL
Thai-US-1-CD163     DCPARSWGHS DCGHKEDAAV TCSEIAKSRE SLHATGRSSF VALAIFGVIL

                    ....|....| ....|....| ....|....| ....|....| ....|....|
                           1060       1070       1080       1090       1100
refCD163            LACLIAFLIW TQKRRQRQRL SVFSGGENSV HQIQYREMNS CLKADETDML
Thai-HP-1-CD163     LACLIAFLIW TQKRRQRQRL SVFSGGENSV HQIQYREMNS CLKADETDML
Thai-EU-1-CD163     LACLIAFLIW TQKRRQRQRL SVFSGGENSV HQIQYREMNS CLKADETDML
Thai-US-1-CD163     LACLIAFLIW TQKRRQRQRL SVFSGGENSV HQIQYREMNS CLKADETDML

                     ....|....| ....|....| ....|....| ...
                            1110       1120       1130
refCD163             NPSGDHSEVQ *KGKWEL*PG EFSL*DTLMK TWT
Thai-HP-1-CD163      NPSGDHSEVQ *KGKWEL*PG EFSL*DTLMK TWT
Thai-EU-1-CD163      NPSGDHSEVQ *KGKWEL*PG EFSL*DTLMK TWT
Thai-US-1-CD163      NPSGDHSEVQ *KGKWEL*PG EFSL*DTLMK TWT
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Figure 4.14 Deduced amino acid alignments of the reference CD163 (refCD163) (GenBank accession number is NP_999141) with recombinant plasmids of Thai-HP-1-pCR®-XL-TOPO®-CD163 (Thai-HP-1-CD163), Thai-EU-1-pCR®-XL-TOPO®-CD163 (Thai-EU-1-CD163) and Thai-US-1-pCR®-XL-TOPO®-CD163 (Thai-US-1-CD163), respectively. The amino acid changes are boxed.

2.4 Nucleotide and deduced amino acid sequences of recombinant porcine CD163 (domain 5) plasmids

Three recombinant plasmids of Thai-HP-1-pCR®8-GW-TOPO®-CD163-DO5 (Thai-HP-1-CD163-DO5), Thai-EU-1-pCR®8-GW-TOPO®-CD163-DO5 (Thai-EU-1-CD163-DO5) and Thai-US-1-pCR®8-GW-TOPO®-CD163-DO5 (Thai-US-1-CD163-DO5) were selected and sequenced. The complete sequences of 3 recombinant porcine CD163 (domain 5) plasmids were successfully sequenced at the same length of 395 bp and were analyzed alignment with the available databases of refCD163 cDNA (accession number is NM_213976) form the alveolar macrophages of normal pigs as shown in Table 4.9 and Figure 4.15, respectively. The results revealed that the nucleotide sequences of 3 recombinant porcine CD163 (domain 5) plasmids were the same with refCD163 cDNA (accession number is NM_213976).

The deduced amino acids sequences and alignment of recombinant porcine CD163 (domain 5) plasmids (131 amino acids) from PRRVS infected PAM with refCD163 (accession number is NP_999141) (131 amino acids) from normal PAM were shown in Table 4.10 and Figure 4.16, respectively. The results revealed that the amino acid sequences of 3 recombinant porcine CD163 (domain 5) plasmids were not different with the same refCD163 cDNA.

Table 4.9 Nucleotide sequences of 3 recombinant porcine CD163 (domain 5) plasmids

Product's name	Sequence
1. Thai-HP-1-CD163-DO5 (395 bp)	ggacattccctgctctggtcgtgttgaagtacaacatggagacacgtggggcaccgtctgtgattct gacttctctctggaggcggccagcgtgctgtgcagggaactacagtgcggcactgtggtttccctc ctgggggggagctcactttggagaaggaagtggacagatctgggctgaagaattccagtgtgagg ggcacgagtcccacctttcactctgcccagtagcaccccgccctgacgggacatgtagccacag cagggacgtcggcgtagtctgctcaagatacacacaaatccgcttggtgaatggcaagacccca tgtgaaggaagagtggagctcaacattcttgggtcctgggggtccctctgcaactctcactgggac atgg
2. Thai-EU-1-CD163-DO5 (395 bp)	ggacattccctgctctggtcgtgttgaagtacaacatggagacacgtggggcaccgtctgtgattct gacttctctctggaggcggccagcgtgctgtgcagggaactacagtgcggcactgtggtttccctc ctgggggggagctcactttggagaaggaagtggacagatctgggctgaagaattccagtgtgagg ggcacgagtcccacctttcactctgcccagtagcaccccgccctgacgggacatgtagccacag cagggacgtcggcgtagtctgctcaagatacacacaaatccgcttggtgaatggcaagacccca tgtgaaggaagagtggagctcaacattcttgggtcctgggggtccctctgcaactctcactgggac atgg
3. Thai-US-1-CD163-DO5 (395 bp)	ggacattccctgctctggtcgtgttgaagtacaacatggagacacgtggggcaccgtctgtgattct gacttctctctggaggcggccagcgtgctgtgcagggaactacagtgcggcactgtggtttccctc ctgggggggagctcactttggagaaggaagtggacagatctgggctgaagaattccagtgtgagg ggcacgagtcccacctttcactctgcccagtagcaccccgccctgacgggacatgtagccacag cagggacgtcggcgtagtctgctcaagatacacacaaatccgcttggtgaatggcaagacccca tgtgaaggaagagtggagctcaacattcttgggtcctgggggtccctctgcaactctcactgggac atgg

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                        ....|....| ....|....| ....|....| ....|....| ....|....|
                                10         20         30         40         50
                          M13 Forward
refCD163                ---------- ---------- ---------- ---------- ----------
Thai-HP-1-CD163-DO5     GTAAAACGAC GGCCAGTCTT AAGCTCGGGC CCCAAATAAT GATTTTATTT
Thai-EU-1-CD163-DO5     GTAAAACGAC GGCCAGTCTT AAGCTCGGGC CCCAAATAAT GATTTTATTT
Thai-US-1-CD163-DO5     GTAAAACGAC GGCCAGTCTT AAGCTCGGGC CCCAAATAAT GATTTTATTT

                        ....|....| ....|....| ....|....| ....|....| ....|....|
                                60         70         80         90        100
refCD163                ---------- ---------- ---------- ---------- ----------
Thai-HP-1-CD163-DO5     TGACTGATAG TGACCTGTTC GTTGCAACAA ATTGATGAGC AATGCTTTTT
Thai-EU-1-CD163-DO5     TGACTGATAG TGACCTGTTC GTTGCAACAA ATTGATGAGC AATGCTTTTT
Thai-US-1-CD163-DO5     TGACTGATAG TGACCTGTTC GTTGCAACAA ATTGATGAGC AATGCTTTTT

                        ....|....| ....|....| ....|....| ....|....| ....|....|
                               110        120        130        140        150
refCD163                ---------- ---------- ---------- ---------- ------GGAC
Thai-HP-1-CD163-DO5     TATAATGCCA ACTTTGTACA AAAAAGCAGG CTCCGAATTC GCCCTTGGAC
Thai-EU-1-CD163-DO5     TATAATGCCA ACTTTGTACA AAAAAGCAGG CTCCGAATTC GCCCTTGGAC
Thai-US-1-CD163-DO5     TATAATGCCA ACTTTGTACA AAAAAGCAGG CTCCGAATTC GCCCTTGGAC

                        ....|....| ....|....| ....|....| ....|....| ....|....|
                               160        170        180        190        200
refCD163                ATTCCCTGCT CTGGTCGTGT TGAAGTACAA CATGGAGACA CGTGGGGCAC
Thai-HP-1-CD163-DO5     ATTCCCTGCT CTGGTCGTGT TGAAGTACAA CATGGAGACA CGTGGGGCAC
Thai-EU-1-CD163-DO5     ATTCCCTGCT CTGGTCGTGT TGAAGTACAA CATGGAGACA CGTGGGGCAC
Thai-US-1-CD163-DO5     ATTCCCTGCT CTGGTCGTGT TGAAGTACAA CATGGAGACA CGTGGGGCAC

                        ....|....| ....|....| ....|....| ....|....| ....|....|
                               210        220        230        240        250
refCD163                CGTCTGTGAT TCTGACTTCT CTCTGGAGGC GGCCAGCGTG CTGTGCAGGG
Thai-HP-1-CD163-DO5     CGTCTGTGAT TCTGACTTCT CTCTGGAGGC GGCCAGCGTG CTGTGCAGGG
Thai-EU-1-CD163-DO5     CGTCTGTGAT TCTGACTTCT CTCTGGAGGC GGCCAGCGTG CTGTGCAGGG
Thai-US-1-CD163-DO5     CGTCTGTGAT TCTGACTTCT CTCTGGAGGC GGCCAGCGTG CTGTGCAGGG

                        ....|....| ....|....| ....|....| ....|....| ....|....|
                               260        270        280        290        300
refCD163                AACTACAGTG CGGCACTGTG GTTTCCCTCC TGGGGGGAGC TCACTTTGGA
Thai-HP-1-CD163-DO5     AACTACAGTG CGGCACTGTG GTTTCCCTCC TGGGGGGAGC TCACTTTGGA
Thai-EU-1-CD163-DO5     AACTACAGTG CGGCACTGTG GTTTCCCTCC TGGGGGGAGC TCACTTTGGA
Thai-US-1-CD163-DO5     AACTACAGTG CGGCACTGTG GTTTCCCTCC TGGGGGGAGC TCACTTTGGA

                        ....|....| ....|....| ....|....| ....|....| ....|....|
                               310        320        330        340        350
refCD163                GAAGGAAGTG GACAGATCTG GGCTGAAGAA TTCCAGTGTG AGGGGCACGA
Thai-HP-1-CD163-DO5     GAAGGAAGTG GACAGATCTG GGCTGAAGAA TTCCAGTGTG AGGGGCACGA
Thai-EU-1-CD163-DO5     GAAGGAAGTG GACAGATCTG GGCTGAAGAA TTCCAGTGTG AGGGGCACGA
Thai-US-1-CD163-DO5     GAAGGAAGTG GACAGATCTG GGCTGAAGAA TTCCAGTGTG AGGGGCACGA

                        ....|....| ....|....| ....|....| ....|....| ....|....|
                               360        370        380        390        400
refCD163                GTCCCACCTT TCACTCTGCC CAGTAGCACC CCGCCCTGAC GGGACATGTA
Thai-HP-1-CD163-DO5     GTCCCACCTT TCACTCTGCC CAGTAGCACC CCGCCCTGAC GGGACATGTA
Thai-EU-1-CD163-DO5     GTCCCACCTT TCACTCTGCC CAGTAGCACC CCGCCCTGAC GGGACATGTA
Thai-US-1-CD163-DO5     GTCCCACCTT TCACTCTGCC CAGTAGCACC CCGCCCTGAC GGGACATGTA
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                    ....|....| ....|....| ....|....| ....|....| ....|....|
                             410        420        430        440        450
refCD163            GCCACAGCAG GGACGTCGGC GTAGTCTGCT CAAGATACAC ACAAATCCGC
Thai-HP-1-CD163-DO5 GCCACAGCAG GGACGTCGGC GTAGTCTGCT CAAGATACAC ACAAATCCGC
Thai-EU-1-CD163-DO5 GCCACAGCAG GGACGTCGGC GTAGTCTGCT CAAGATACAC ACAAATCCGC
Thai-US-1-CD163-DO5 GCCACAGCAG GGACGTCGGC GTAGTCTGCT CAAGATACAC ACAAATCCGC

                    ....|....| ....|....| ....|....| ....|....| ....|....|
                             460        470        480        490        500
refCD163            TTGGTGAATG GCAAGACCCC ATGTGAAGGA AGAGTGGAGC TCAACATTCT
Thai-HP-1-CD163-DO5 TTGGTGAATG GCAAGACCCC ATGTGAAGGA AGAGTGGAGC TCAACATTCT
Thai-EU-1-CD163-DO5 TTGGTGAATG GCAAGACCCC ATGTGAAGGA AGAGTGGAGC TCAACATTCT
Thai-US-1-CD163-DO5 TTGGTGAATG GCAAGACCCC ATGTGAAGGA AGAGTGGAGC TCAACATTCT

                    ....|....| ....|....| ....|....| ....|....| .
                             510        520        530        540
refCD163            TGGGTCCTGG GGGTCCCTCT GCAACTCTCA CTGGGACATG G
Thai-HP-1-CD163-DO5 TGGGTCCTGG GGGTCCCTCT GCAACTCTCA CTGGGACATG G
Thai-EU-1-CD163-DO5 TGGGTCCTGG GGGTCCCTCT GCAACTCTCA CTGGGACATG G
Thai-US-1-CD163-DO5 TGGGTCCTGG GGGTCCCTCT GCAACTCTCA CTGGGACATG G
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Figure 4.15 Nucleotide sequence alignments of the reference CD163 (refCD163) (accession number is NM_213976) with recombinant plasmids of Thai-HP-1-pCR®8-GW-TOPO®-CD163-DO5 (Thai-HP-1-CD163-DO5), Thai-EU-1-pCR®8-GW-TOPO®-CD163-DO5 (Thai-EU-1-CD163-DO5) and Thai-US-1-pCR®8-GW-TOPO®-CD163-DO5 (Thai-US-1-CD163-DO5), respectively. The nucleotide change is boxed.

Table 4.10 Deduced amino acid sequences of 3 recombinant porcine CD163 (domain 5) plasmids

Product's name	Sequence
1. Thai-HP-1-CD163-DO5 (131 aa)	GHSLLWSC*STTWRHVGHRL*F*LLSGGGQRAVQGTTVRHCGFPPGGSSLWR RKWTDLG*RIPV*GARVPPFTLPSSTPP*RDM*PQQGRRRSLLKIHTNPLGEWQ DPM*RKSGAQHSWVLGVPLQLSLGH
2. Thai-EU-1-CD163-DO5 (131 aa)	GHSLLWSC*STTWRHVGHRL*F*LLSGGGQRAVQGTTVRHCGFPPGGSSLWR RKWTDLG*RIPV*GARVPPFTLPSSTPP*RDM*PQQGRRRSLLKIHTNPLGEWQ DPM*RKSGAQHSWVLGVPLQLSLGH
3. Thai-US-1-CD163-DO5 (131 aa)	GHSLLWSC*STTWRHVGHRL*F*LLSGGGQRAVQGTTVRHCGFPPGGSSLWR RKWTDLG*RIPV*GARVPPFTLPSSTPP*RDM*PQQGRRRSLLKIHTNPLGEWQ DPM*RKSGAQHSWVLGVPLQLSLGH

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                    ....|....| ....|....| ....|....| ....|....| ....|....|
                             10         20         30         40         50
refCD163            GHSLLWSC*S TTWRHVGHRL *F*LLSGGGQ RAVQGTTVRH CGFPPGGSSL
Thai-HP-1-CD163-DO5 GHSLLWSC*S TTWRHVGHRL *F*LLSGGGQ RAVQGTTVRH CGFPPGGSSL
Thai-EU-1-CD163-DO5 GHSLLWSC*S TTWRHVGHRL *F*LLSGGGQ RAVQGTTVRH CGFPPGGSSL
Thai-US-1-CD163-DO5 GHSLLWSC*S TTWRHVGHRL *F*LLSGGGQ RAVQGTTVRH CGFPPGGSSL

                    ....|....| ....|....| ....|....| ....|....| ....|....|
                             60         70         80         90        100
refCD163            WRRKWTDLG* RIPV*GARVP PFTLPSSTPP *RDM*PQQGR RRSLLKIHTN
Thai-HP-1-CD163-DO5 WRRKWTDLG* RIPV*GARVP PFTLPSSTPP *RDM*PQQGR RRSLLKIHTN
Thai-EU-1-CD163-DO5 WRRKWTDLG* RIPV*GARVP PFTLPSSTPP *RDM*PQQGR RRSLLKIHTN
Thai-US-1-CD163-DO5 WRRKWTDLG* RIPV*GARVP PFTLPSSTPP *RDM*PQQGR RRSLLKIHTN

                    ....|....| ....|....| ....|....| .
                            110        120        130
refCD163            PLGEWQDPM* RKSGAQHSWV LGVPLQLSLG H
Thai-HP-1-CD163-DO5 PLGEWQDPM* RKSGAQHSWV LGVPLQLSLG H
Thai-EU-1-CD163-DO5 PLGEWQDPM* RKSGAQHSWV LGVPLQLSLG H
Thai-US-1-CD163-DO5 PLGEWQDPM* RKSGAQHSWV LGVPLQLSLG H
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Figure 4.16 Deduced amino acid alignments of the reference CD163 (refCD163) (accession number is NP_999141) with recombinant plasmids of Thai-HP-1-pCR®8-GW-TOPO®-CD163-DO5 (Thai-HP-1-CD163-DO5), Thai-EU-1-pCR®8-GW-TOPO®-CD163-DO5 (Thai-EU-1-CD163-DO5) and Thai-US-1-pCR®8-GW-TOPO®-CD163-DO5 (Thai-US-1-CD163-DO5), respectively. The amino acid change is boxed.

3. Nucleotide and deduced amino acid compositions of recombinant porcine Sn and CD163 plasmids

3.1 Nucleotide compositions of recombinant porcine Sn plasmids

The nucleotide compositions of recombinant porcine Sn (full and N-terminal domain) plasmids were shown as in Table 4.11. The results indicated that the number of the Cytosine nucleotide of porcine Sn (full) from 3 recombinant plasmids of Thai-HP-1-pCR®-XL-TOPO®-Sn (Thai-HP-1-Sn), Thai-EU-1-pCR®-XL-TOPO®-Sn (Thai-EU-1-Sn), Thai-US-1-pCR®-XL-TOPO®-Sn (Thai-US-1-Sn) and the refSn cDNA from normal PAM (accession number is NM_214346) was higher than that of the other nucleotides.

The number of Cytosine nucleotide compositions in porcine Sn (N-terminal domain) of 3 recombinant plasmids, Thai-HP-1-pCR®8-GW-TOPO®-N-terminal Sn (Thai-HP-1-N-terminal-Sn), Thai-EU-1-pCR®8-GW-TOPO®-N-terminal-Sn (Thai-EU-1-N-terminal-Sn) and Thai-US-1-pCR®8-GW-TOPO®-N-terminal-Sn (Thai-US-1-N-terminal-Sn), from PRRSV infected PAM and the refSn cDNA from normal PAM was higher than that of the remaining nucleotides.

Table 4.11 Nucleotide compositions of recombinant porcine Sn plasmids

Nucleotide	Sn							
	Number of nucleotide							
	Full				N-terminal domain			
	refSn	Thai-HP-1	Thai-EU-1	Thai-US-1	refSn	Thai-HP-1	Thai-EU-1	Thai-US-1
A	924	923	926	923	203	202	203	202
C	1,803	1,806	1,801	1,804	336	337	336	337
G	1,486	1,485	1,485	1,486	293	292	293	292
T	980	979	981	980	192	193	192	193
Total	5,193	5,193	5,193	5,193	1,024	1,024	1,024	1,024

Note: (A: Adenine; C: Cytosine; G: Guanine; T: Thymine)

3.2 Deduced amino acid compositions of recombinant porcine Sn plasmids

The deduced amino acid compositions of recombinant porcine Sn (full and N-terminal domain) plasmids were shown in Table 4.12. The number of Leucine, Alanine and Serine of 3 recombinant porcine Sn (full) plasmids, Thai-HP-1-pCR®-XL-TOPO®-Sn (Thai-HP-1-Sn), Thai-EU-1-pCR®-XL-TOPO®-Sn (Thai-EU-1-Sn) and Thai-US-1-pCR®-XL-TOPO®-Sn (Thai-US-1-Sn), and the refSn from normal PAM (accession number is NP_999511) was higher than that of the other amino acids.

The results of deduced amino acid compositions of recombinant porcine Sn (N-terminal domain) plasmids revealed that the number of the Valine, Leucine and Serine from 3 recombinant porcine Sn (N-terminal domain) plasmids, Thai-HP-1-pCR®8-GW-TOPO®-N-terminal Sn (Thai-HP-1-N-terminal-Sn), Thai-EU-1-pCR®8-GW-TOPO®-N-terminal-Sn (Thai-EU-1-N-terminal-Sn) and Thai-US-1-pCR®8-GW-TOPO®-N-terminal-Sn (Thai-US-1-N-terminal-Sn), and the refSn from normal PAM (accession number is NP_999511) was higher than that of the remaining amino acids.

Table 4.12 Deduced amino acid compositions of recombinant porcine Sn plasmids

Amino acids	Abbreviation		Sn							
			Number of amino acids							
			Full				N-terminal domain			
			refSn	Thai-HP-1	Thai-EU-1	Thai-US-1	refSn	Thai-HP-1	Thai-EU-1	Thai-US-1
Alanine	Ala	A	183	183	183	183	20	20	20	20
Cysteine	Cys	C	41	41	41	41	8	9	8	9
Aspartate	Asp	D	65	65	65	65	12	12	11	12
Glutamate	Glu	E	80	80	80	80	17	17	17	17
Phenylalanine	Phe	F	36	36	36	36	9	9	9	9
Glycine	Gly	G	124	123	124	123	23	22	23	22
Histidine	His	H	52	52	52	52	11	12	12	12
Isoleucine	Ile	I	34	34	34	34	11	11	11	11
Lysine	Lys	K	27	27	27	27	13	13	13	13
Leucine	Leu	L	219	218	219	218	36	36	35	36
Methionine	Met	M	20	20	20	20	6	6	6	6
Asparagine	Asn	N	50	50	50	50	12	12	12	12
Proline	Pro	P	111	111	111	111	20	20	20	20
Glutamine	Gln	Q	91	91	91	91	23	23	23	23
Arginine	Arg	R	97	98	97	98	14	15	14	15
Serine	Ser	S	173	174	173	174	36	35	35	35
Threonine	Thr	T	117	117	117	117	19	19	19	19
Valine	Val	V	145	145	145	145	37	37	38	37
Tryptophan	Trp	W	25	25	25	25	7	7	7	7
Tyrosine	Tyr	Y	40	40	40	40	7	6	7	6
Total			1,730	1,730	1,730	1,730	341	341	341	341

3.3 Nucleotide compositions of recombinant porcine CD163 plasmids

The nucleotide compositions of recombinant porcine CD163 (full and domain 5) plasmids were shown as in Table 4.13. The results indicated that the numbers of the Guanine nucleotide of 3 recombinant porcine CD163 (full) plasmids, Thai-HP-1-pCR®-XL-TOPO®-CD163 (Thai-HP-1-CD163), Thai-EU-1-pCR®-XL-TOPO®-CD163 (Thai-EU-1-CD163), and Thai-US-1-pCR®-XL-TOPO®-CD163 (Thai-US-1-CD163), and the refCD163 cDNA from normal PAM (accession number is NM_213976) was the highest number. In contrast, the numbers of the Cytosine nucleotide was the lowest number.

The results of nucleotide compositions of recombinant porcine CD163 (domain 5) plasmids revealed that the numbers of the Guanine nucleotide from 3 recombinant porcine CD163 (domain 5) plasmids, Thai-HP-1-pCR®8-GW-TOPO®-CD163-DO5 (Thai-HP-1-CD163-DO5), Thai-EU-1-pCR®8-GW-TOPO®-CD163-DO5 (Thai-EU-1-CD163-DO5) and Thai-US-1-pCR®8-GW-TOPO®-CD163-DO5 (Thai-US-1-CD163-DO5), and the refCD163 cDNA normal PAM (accession number is NM_213976) was the highest numbers and followed by the number of Cytosine nucleotide.

Table 4.13 Nucleotide compositions of recombinant porcine CD163 plasmids

Nucleotide	CD163							
	Number of nucleotide							
	Full				N-terminal domain			
	refCD163	Thai-HP-1	Thai-EU-1	Thai-US-1	refCD163	Thai-HP-1	Thai-EU-1	Thai-US-1
A	867	868	867	867	83	83	83	83
C	687	685	685	686	104	104	104	104
G	1,018	1,019	1,021	1,019	123	123	123	123
T	828	828	827	826	85	85	85	85
Total	3,400	3,400	3,400	3,400	395	395	395	395

Note: (A: Adenine; C: Cytosine; G: Guanine; T: Thymine)

3.4 Deduced amino acid compositions of recombinant porcine CD163 plasmids

The deduced amino acid compositions of recombinant porcine CD163 (full and domain 5) plasmids were shown as in Table 4.14. The number of the Glycine and Serine amino acids of 3 recombinant porcine CD163 (full) plasmids, Thai-HP-1-pCR®-XL-TOPO®-CD163 (Thai-HP-1-CD163), Thai-EU-1-pCR®-XL-TOPO®-CD163 (Thai-EU-1-CD163), and Thai-US-1-pCR®-XL-TOPO®-CD163 (Thai-US-1-CD163), and the refCD163 from normal PAM (accession number is NP_999141) was higher than that of the other amino acids.

The results of deduced amino acid compositions of recombinant porcine CD163 (domain 5) plasmids revealed that the number of the Glycine and Leucine amino acid from 3 recombinant porcine CD163 (domain 5) cDNA plasmids, Thai-HP-1-pCR®8-GW-TOPO®-CD163-DO5 (Thai-HP-1-CD163-DO5), Thai-EU-1-pCR®8-GW-TOPO®-CD163-DO5 (Thai-EU-1-CD163-DO5) and Thai-US-1-pCR®8-GW-TOPO®-CD163-DO5 (Thai-US-1-CD163-DO5), and the refCD163 from normal PAM (accession number is NP_999141) was higher than that of the remaining amino acids.

Table 4.14 Deduced amino acid compositions of recombinant porcine CD163 plasmids

Amino acids	Abbreviation		CD163							
			Number of amino acids							
			Full				Domain 5			
			ref CD163	Thai-HP-1	Thai-EU-1	Thai-US-1	ref CD163	Thai-HP-1	Thai-EU-1	Thai-US-1
Alanine	Ala	A	72	73	72	73	3	3	3	3
Cysteine	Cys	C	76	75	76	76	2	2	2	2
Aspartate	Asp	D	66	65	65	65	3	3	3	3
Glutamate	Glu	E	70	70	71	70	1	1	1	1
Phenylalanine	Phe	F	25	26	25	25	3	3	3	3
Glycine	Gly	G	130	131	131	130	16	16	16	16
Histidine	His	H	42	42	42	42	7	7	7	7
Isoleucine	Ile	I	38	38	38	38	2	2	2	2
Lysine	Lys	K	47	47	47	47	3	3	3	3
Leucine	Leu	L	81	80	80	80	15	15	15	15
Methionine	Met	M	13	13	13	13	2	2	2	2
Asparagine	Asn	N	46	46	46	46	1	1	1	1
Proline	Pro	P	28	28	29	28	12	12	12	12
Glutamine	Gln	Q	40	40	40	40	7	7	7	7
Arginine	Arg	R	52	51	51	52	13	13	13	13
Serine	Ser	S	115	114	114	115	12	12	12	12
Threonine	Thr	T	51	51	51	51	8	8	8	8
Valine	Val	V	84	85	85	85	7	7	7	7
Tryptophan	Trp	W	45	45	45	45	6	6	6	6
Tyrosine	Tyr	Y	9	9	9	9	0	0	0	0
Total			1,133	1,133	1,133	1,133	131	131	131	131

4. The homology of nucleotide and deduced amino acid sequences of recombinant porcine Sn and CD163 plasmids

4.1 The homology of nucleotide and deduced amino acid sequences of 3 recombinant porcine Sn (full) plasmids

The recombinant porcine Sn (full) plasmids of Thai-HP-1-pCR®-XL-TOPO®-Sn (Thai-HP-1-Sn), Thai-EU-1-pCR®-XL-TOPO®-Sn (Thai-EU-1-Sn) and Thai-US-1-pCR®-XL-TOPO®-Sn (Thai-US-1-Sn) were further compared with the refSn cDNA from GenBank (accession number is NM_214346) by using Bioedit Sequence Alignment Editor software. The percentage homology among those recombinant plasmids and refSn database were demonstrated in Table 4.15. The results showed high nucleotide homology at 99.8 - 99.9 % identities. The deduced amino acid sequences revealed that among those recombinant plasmids, Thai-HP-1-pCR®-XL-TOPO®-Sn, Thai-EU-1-pCR®-XL-TOPO®-Sn and Thai-US-1-pCR®-XL-TOPO®-Sn, were similar at 99.8 - 100 % amino acid identities.

Table 4.15 Comparison homology (%) of nucleotide and deduced amino acid sequences of the refSn and 3 recombinant porcine Sn (full) plasmids (Nucleotide identity (%) in lower triangle of table; Deduced amino acid identity (%) in upper triangle of table)

Sequences	refSn	Thai-HP-1-Sn	Thai-EU-1-Sn	Thai-US-1-Sn
refSn		99.8	100	99.8
Thai-HP-1-Sn	99.8		99.8	100
Thai-EU-1-Sn	99.8	99.8		99.8
Thai-US-1-Sn	99.8	99.9	99.8	

4.2 The homology of nucleotide and deduced amino acid sequences of recombinant porcine Sn (N-terminal domain) plasmids

The recombinant porcine Sn (N-terminal domain) plasmids of Thai-HP-1-pCR®8-GW-TOPO®-N-terminal-Sn (Thai-HP-1-N-terminal-Sn), Thai-EU-1-pCR®8-GW-TOPO®-N-terminal-Sn (Thai-EU-1-N-terminal-Sn) and Thai-US-1-pCR®8-GW-TOPO®-N-terminal-Sn (Thai-US-1-N-terminal-Sn) were compared with refSn from GenBank (accession number is NM_214346) by using Bioedit Sequence Alignment Editor software. The number homology among these recombinant porcine Sn (N-terminal domain) plasmids, Thai-HP-1-pCR®8-GW-TOPO®-N-terminal-Sn, Thai-EU-1-pCR®8-GW-TOPO®-N-terminal-Sn and Thai-US-1-pCR®8-GW-TOPO®-N-terminal-Sn, and refSn were shown in Table 4.16. The results showed high nucleotide identities at 99.8 - 100 % homology. The deduced amino acid sequences revealed that the recombinant porcine Sn (N-terminal domain) plasmids, Thai-HP-1-pCR®8-GW-TOPO®-N-terminal-Sn, Thai-EU-1-pCR®8-GW-TOPO®-N-terminal-Sn and Thai-US-1-pCR®8-GW-TOPO®-N-terminal-Sn, were similar at 99.7 - 100 % of amino acid identities.

Table 4.16 Comparison homology (%) of nucleotide and deduced amino acid sequences of the refSn and 3 recombinant porcine Sn (N-terminal domain) plasmids (Nucleotide identity (%) in lower triangle of table; Deduced amino acid identity (%) in upper triangle of table)

Sequences	refSn	Thai-HP-1-N-terminal-Sn	Thai-EU-1-N-terminal-Sn	Thai-US-1-N-terminal-Sn
refSn		99.7	100	99.7
Thai-HP-1-N-terminal-Sn	99.8		99.7	100
Thai-EU-1-N-terminal-Sn	100	99.8		99.7
Thai-US-1-N-terminal-Sn	99.8	100	99.8	

4.3 The homology of nucleotide and deduced amino acid sequences of recombinant porcine CD163 (full) plasmids

The recombinant porcine CD163 (full) plasmids of Thai-HP-1-pCR®-XL-TOPO®-CD163 (Thai-HP-1-CD163), Thai-EU-1-pCR®-XL-TOPO®-CD163 (Thai-EU-1-CD163), and Thai-US-1-pCR®-XL-TOPO®-CD163 (Thai-US-1-CD163), were further analyzed with refCD163 (refCD163) from GenBank (accession number is NM_213976) by using Bioedit Sequence Alignment Editor software. The homology among these recombinant porcine CD163 (full) plasmids, Thai-HP-1-pCR®-XL-TOPO®-CD163, Thai-EU-1-pCR®-XL-TOPO®-CD163 and Thai-US-1-pCR®-XL-TOPO®-CD163, and refCD163 were shown in Table 4.17. The results of nucleotide sequences revealed high percent homology at 99.7 - 99.8 %

homology. The deduced amino acid sequences revealed that the recombinant porcine CD163 (full) plasmids of Thai-HP-1-pCR®-XL-TOPO®-CD163, Thai-EU-1-pCR®-XL-TOPO®-CD163 and Thai-US-1-pCR®-XL-TOPO®-CD163 were similar at 99.5, 99.6 and 99.7 % of amino acid identities, respectively.

Table 4.17 Comparison homology (%) of nucleotide and deduced amino acid sequences of the refCD163 and 3 recombinant porcine CD163 (full) plasmids (Nucleotide identity (%) in lower triangle of table; Deduced amino acid identity (%) in upper triangle of table)

Sequences	refCD163	Thai-HP-1-CD163	Thai-EU-1-CD163	Thai-US-1-CD163
refCD163		99.5	99.6	99.8
Thai-HP-1-CD163	99.7		99.5	99.7
Thai-EU-1-CD163	99.7	99.8		99.6
Thai-US-1-CD163	99.8	99.8	99.8	

4.4 The homology of nucleotide and deduced amino acid sequences of recombinant porcine CD163 (domain 5) cDNA plasmids

The recombinant porcine CD163 (domain 5) plasmids of Thai-HP-1-pCR®8-GW-TOPO®-CD163-DO5 (Thai-HP-1-CD163-DO5), Thai-EU-1-pCR®8-GW-TOPO®-CD163-DO5 (Thai-EU-1-CD163-DO5) and Thai-US-1-pCR®8-GW-TOPO®-CD163-DO5 (Thai-US-1-CD163-DO5) were compared with refCD163 from GenBank (accession number is NM_213976) using Bioedit Sequence Alignment Editor software. The results of nucleotide sequences revealed at 100 % homology. The number homology of deduced amino acid among these

recombinant porcine CD163 (domain 5) plasmids of Thai-HP-1-pCR®8-GW-TOPO®-CD163-DO5, Thai-EU-1-pCR®8-GW-TOPO®-CD163-DO5 and Thai-US-1-pCR®8-GW-TOPO®-CD163-DO5 and refCD163 were shown in Table 4.18. The results revealed high deduced amino acid at 100 % identities.

Table 4.18 Comparison homology (%) of nucleotide and deduced amino acid sequences of refCD163 and 3 recombinant porcine CD163 (domain 5) plasmids (Nucleotide identity (%) in lower triangle of table; Deduced amino acid identity (%) in upper triangle of table)

Sequences	refCD163	Thai-HP-1-CD163-DO5	Thai-EU-1-CD163-DO5	Thai-US-1-CD163-DO5
refCD163		100	100	100
Thai-HP-1-CD163-DO5	100		100	100
Thai-EU-1-CD163-DO5	100	100		100
Thai-US-1-CD163-DO5	100	100	100	

CHAPTER V

DISCUSSION

PRRS has caused severe economic loss in most swine-producing countries. For a basic research on pathogenesis of PRRSV infection in nursery pigs, porcine Sn and CD163 which have been identified as the major receptors for PRRSV (Calvert et al., 2007; Van Gorp et al., 2008) infection on the macrophages were studied. In this thesis, the author first reported in Thailand about the cloning using RT-PCR technique, the expression level using gel densitometry, the genetic and deduced amino acid sequences and both compositions of the porcine Sn (full and N-terminal domain) and CD163 (full and domain 5) on alveolar macrophages of nursery pigs infected with 3 strains of PRRSV. The author also demonstrated the homology and the comparison of both receptor data among the 3 PRRSV strains.

In general, there are many methods to study the comparison of the gene expression level including gel densitometry (Dozois et al., 1997; Schmittgen et al., 2000; Etienne et al., 2004). The author selected the GAPDH gene as internal controls for levels of housekeeping genes because this gene was usually used for RT-PCR analysis in human tissues (Suzuki et al., 2000; Sila-Asna et al., 2007) and porcine tissues (Foss et al., 1998). The author found that US strain of PRRSV infected PAM samples were showed higher expression level of both porcine Sn (full and N-terminal domain) and CD163 (full and domain 5) than that of EU and HP strains of PRRSV infected PAM. This finding in part suggested that higher expression of porcine Sn and CD163 were higher the susceptibility to PRRSV infection and the US strain may be easily infected than the others strains (Jiang et al., 2012). In addition, an increase in the percentage of infected cells was correlated with an enhanced expression CD163 of porcine monocytes/macrophages in the process of ASFV infection. The study of Patton et al. (2009) showed that the expression level of CD163 was also correlated well with the overall

level of PRRSV replication. Moreover, the high expression level of human Sn receptor was relatively enhanced HIV infection and infectivity (Rempe et al., 2008). In addition, the infection study of PRRSV in the Chinese Dapulian (DPL) pigs were showed the lower gene expression level of porcine Sn and CD163 on macrophages, the lower rectal temperature and the lower PRRSV viral copy number than that of the commercial Duroc x Landrace x Yorkshire (DLY) crossbred pig indicated that DPL pigs were more resistant than DLY pigs (Jiang et al., 2012). In this thesis, the PAM samples were obtained from the PRRSV outbreak farms and the field data may have many factors involved. According to the limitation of PAM samples from the farms in Ratchaburi province only, the samples from other pig farms from different parts of Thailand should be further studied. The viremic PRRSV in sera samples of Thai nursery pigs should not only confirm the positive with S/P ELISA and RT-PCR, but also quantitatively measure the viral mRNA concentration by using Real-time PCR technique. Moreover, the correlation between the level of receptor expression and the efficiency of PRRSV infection among 3 strains is necessary to obtain a complete study. Furthermore, the comparison study of two receptor gene expressions between normal PAM and PAM infected with PRRSV should be more elucidated.

For an application of these receptors in enhancement of the PRRS vaccine development, porcine Sn and CD163 cDNA were first amplified and cloned into the recombinant plasmids. In this study, the porcine Sn (full and N-terminal domain) and CD163 (full and domain 5) cDNA were cloned into pCR®8-GW-TOPO® vectors. However, only two short fragments of the porcine Sn (N-terminal domain) and CD163 (domain 5) were transformed with positive colonies but the porcine Sn (full) and CD163 (full) were not successfully transformed (no positive colony). Finally, the author has successfully transformed the porcine Sn (full) and CD163 (full) cDNA into pCR®-XL-TOPO® vectors. These recombinant plasmids were useful for study and analysis of the genetic and deduced amino acid sequences and composition of those receptors. There are many factors concerning in selecting the cloning and expression system depending on the biological

properties of interested proteins, the requirement of an amount of recombinant proteins, and the nature of the experiments (Geisse et al., 1996). The porcine Sn and CD163 cDNA were studied in many expression systems (Vanderheijden et al., 2003; Calvert et al., 2007; Pérez et al., 2008). A wide variety of animal receptors were expressed in many expression vectors such as yeast and baculovirus (Tate et al., 2003), whereas and *E.coli* bacterial expressing system was selected because it is cheap, fast and easy to manipulate.

In this thesis, the full length of porcine Sn and CD163 cDNA from 3 strains of PRRSV infected PAM in Thai nursery pigs were completely sequenced and reported at the first time. The composition and homology of nucleotide and deduced amino acid sequences were analyzed. The composition and the homology were also revealed highly conserved in the sequences of porcine Sn and CD163. In addition, the variability of nucleotide and deduced amino acid sequences of porcine Sn and CD163 cDNA from PRRSV infected PAM were not different among 3 strains. The nucleotide and deduced amino acid sequences from recombinant plasmids of porcine Sn and CD163 from 3 strains of PRRSV infected PAM samples revealed no different from the refSn and refCD163 from normal PAM samples. This study showed the same results as the studies by Srikumaran (2006) about Sn and Pérez et al. (2008) about CD163. Both researchers showed that the complete sequences of porcine Sn (Srikumaran, 2006) and CD163 (Pérez et al., 2008) cDNA from PAM samples of healthy pigs by cloning into mammalian system revealed the homology with both receptor ancestors. These results indicated that porcine Sn and CD163 cDNA is the conserved genes. Moreover, the author investigated the domain of porcine Sn that is involved in the interaction with PRRSV. The sialic acid-binding activity of porcine Sn by site-directed mutagenesis and whether the absence of sialic acid-binding activity may be effective the interaction of the porcine arterivirus with Sn (Delputte et al., 2007). The N-terminal domain of Sn was considered to be involved in PRRSV attachment to macrophage cells. Furthermore, An et al. (2010) constructed a series of truncated fragments of porcine Sn and expressed them in the non-permissive PK 15 cell line to investigate whether the N-terminal domain of

Sn is sufficient for PRRSV attachment. Their result showed that the first 150 amino acids comprising the entire first domain of the porcine Sn (N-terminal region) was necessary for PRRSV binding to cells, and the N-terminal domain alone was sufficient for virus attachment. The study of CD163 protein domains involved in PRRSV infection, Van Gorp et al. (2009) created CD163 deletion mutants and chimeric mutants (replacement experiments) and found that scavenger receptor cysteine-rich (SRCR) domain 5 (SRCR 5) consisted of 100 amino acids is essential for PRRSV infection. In this thesis, the genetic and deduced amino acids sequences of porcine Sn (N-terminal domain) and CD163 (domain 5) were investigated. The porcine Sn (N-terminal domain) and CD163 (domain 5) were successfully amplified and the sequence homology was then analyzed. The author demonstrated that the recombinant plasmids of porcine Sn (N-terminal domain) and CD163 (domain 5) of 3 strains of PRRSV infected PAM from Thai nursery pigs were showed the same patterns of the sequences and composition of nucleotide and deduced amino acids sequences as the refSn or refCD163 of PAM isolated from normal pigs. No mutation of deduced amino acid from two receptors (Sn and CD163) among 3 strains of PRRSV was detected in this study. However, the PRRSV infected PAM samples collected from different pig farms of Thailand should be more studied. In this thesis, the data about the composition and deduce amino acid of receptors from 3 strains of PRRSV showed no mutation. This finding will be useful for the development of monoclonal anti-receptor antibody, antiviral drugs or SiRNA to block their cellular receptors in the future (Calvez et al., 2004). However, the PRRSV controlling strategy is still difficult as the same as HIV (Human Immunodeficiency Virus) in human. Many factors or co-factors involved in the PRRSV pathogenesis in pigs such as mutation or variation between host receptors and virus invasion are required additional studies.

Taken together, gained knowledge in this thesis may be useful for further expression and production of these recombinant porcine Sn and CD163 proteins. These results also provide a preliminary data about the sequences and composition of nucleotide and deduce amino acid of both porcine Sn and CD163 after PRRSV infected PAM in Thai nursery pigs

and allow to understand more on characterization of porcine Sn (N-terminal domain) and CD163 (domain 5). The obtained data of the expression level of porcine Sn and CD163 cDNA among three PRRSV strains infected PAM may be contributed the understanding on the inhibition of PRRS virus and the control of PRRS virus infection in the future.

Conclusion

This thesis, the author performed the study of molecular biology of porcine Sn (full and N-terminal domain) and CD163 (full and domain 5) cDNA from PRRSV infected PAM. The amplicons of those porcine Sn and CD163 cDNA were successfully amplified using RT-PCR technique. Furthermore, the author also successfully cloned and constructed the recombinant plasmids of porcine Sn (full and N-terminal domain) and CD163 (full and domain 5) vectors. The results from this thesis showed that the alignment and composition of nucleotide and deduced amino acid sequences from porcine Sn and CD163 cDNA of 3 strains of PRRSV infected PAM are the same. No mutation of deduced amino acid was observed. The obtained knowledge and tools in this study contributes to the basic research of porcine Sn and CD163 expression level, the basic data of nucleotides and deduced amino acid.

For future work, since the applied research for improving the susceptible cell lines to PRRSV infection and for enhancing vaccines production, so recombinant porcine Sn and CD163 expression vectors are further constructed and the expression, purification and reactivity of these recombinant proteins are further studied.

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APPENDICES

APPENDIX A

Instrument and chemical substances

1. A -20°C refrigerator, Model SF-C997 (Sanyo, Thailand)
2. A -80°C refrigerator, Model 905 (Thermofisher Scientific, USA)
3. Centrifuge and Microcentrifuge
4. Experimental glasswares
5. Gel document system, Model GVM 20 (Synoptics, UK)
6. Gel electrophoresis system, Model GE-100 (Bioer technology Co. Ltd., China)
7. Heat block (Labnet International Inc., USA)
8. Incubator, Model BE-400 (Memmert Inc., Germany)
9. Lamina air flow, Model Bio II A (Telstar, Spain)
10. Micropipette (Labnet, USA) and Micropipette tips
11. PCR assay

11.1 Agarose gel (Molecular grade)

11.2 1kb DNA marker

11.3 1kb DNA marker (BioLab, USA)

11.4 Ethidium bromide 10mg/ml (Sigma Aldrich Inc., USA)

11.5 Gel electrophoresis buffer (TAE)

11.6 Loading dye (Fermantas, Canada)

11.7 Itaq DNA polymerase (iNtRon, South Korea)

12. PCR cabinet (Biometra, Germany)
13. PCR tube and Microcentrifuge tube
14. Vortex, Model K 550-GE (Scientific Inc., USA)
15. Shaking incubator, Model 311DS (Labnet International Inc., USA)

APPENDIX B

Reagents and preparations

Reagents for agarose gel electrophoresis

1. 10 mg/ml Ethidium bromide
   - Ethidium bromide 1 g
   - Distilled deionized water 100 ml

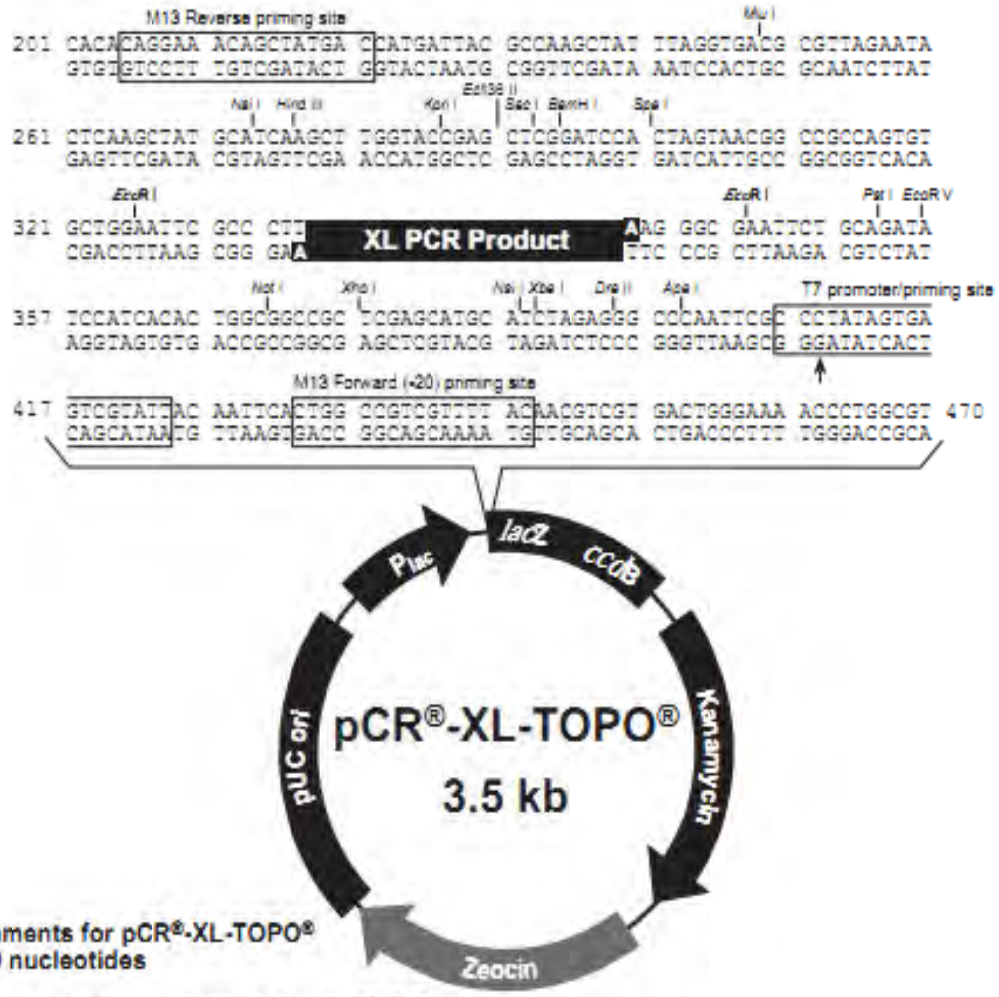
Add 1g of ethidium bromide to 100 ml of distill deionized water. Stir on a magnetic stirrer for several hours to ensure that the dye has dissolved. Wrap container in aluminum foil or transfer to a dark bottle and store at room temperature.

2. 25X TAE (Tris-Acetate buffer) 1,000 ml contains
   - Tris base 242.0 g
   - Glacial acetic acid 57.1 ml
   - 0.5 M EDTA pH 8.0 100.0 ml
   - Distilled deionized water (final volume) 1,000 ml

Add 242 g of Tris base, 57.1 ml of Glacial acetic acid and 100 ml of 0.5 M EDTA pH 8.0 to 500 ml of distilled deionized water and then adjusts the final volume to 1,000 ml. Sterilize the solution by autoclaving.

APPENDIX C

Physical map of plasmid pCR®-XL-TOPO®



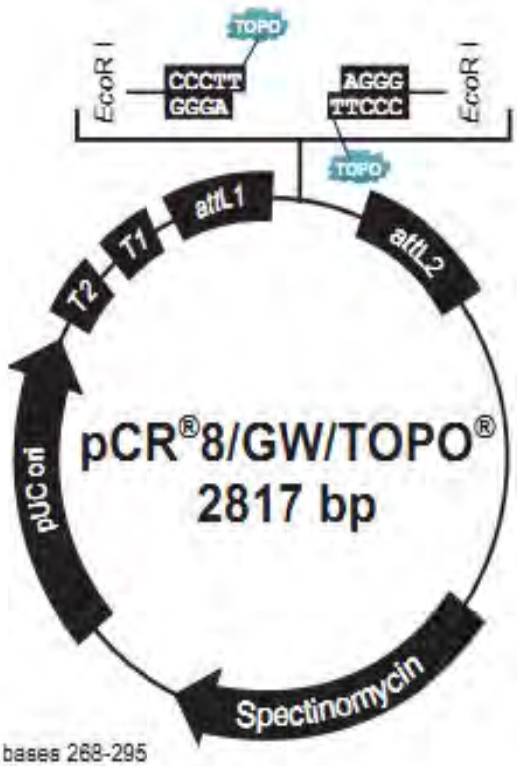
Comments for pCR®-XL-TOPO®
3519 nucleotides

Lac promoter/operator region: bases 95-216
M13 Reverse priming site: bases 205-221
Lac Za ORF: bases 217-576
Multiple Cloning Site: bases 248-399
TOPO® Cloning site: bases 336-337
T7 promoter priming site: bases 406-425
M13 Forward (-20) priming site: bases 433-448
Fusion joint: bases 577-585
*ccd*B lethal gene ORF: bases 586-888
Kanamycin resistance ORF: bases 1237-2031
Zeocin resistance ORF: bases 2238-2612
pUC origin: bases 2680-3393

invitrogen™

APPENDIX D

Physical map of plasmid pCR®8/GW/TOPO®



Comments for pCR®8/GW/TOPO®
2817 nucleotides

*rrn*B T2 transcription termination sequence: bases 268-295
*rrn*B T1 transcription termination sequence: bases 427-470
M13 forward (-20) priming site: bases 537-552
attL1: bases 569-668
GW1 priming site: bases 607-631
TOPO® recognition site 1: bases 678-682
TOPO® recognition site 2: bases 683-687
attL2: bases 696-795
GW2 priming site: bases 733-757
T7 Promoter/priming site: 812-831 (c)
M13 reverse priming site: bases 836-852
Spectinomycin promoter: bases 930-1063
Spectinomycin resistance gene (Spn^R): 1064-2074
pUC origin: bases 2141-2814

(c) = complementary sequence

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BIOGRAPHY

Mr. Vo Phong Vu Anh Tuan was born on 19 September 1978 in Chogao district, Tiengiang province, Vietnam. He is doctor of veterinary medicine, graduated from Faculty of Animal Science and Veterinary Medicine, Agriculture and Forestry University, Ho Chi Minh city, Vietnam in 2002. After graduation, he works as lecturer of major domesticated animal disease in department of Veterinary Medicine, Faculty of Animal Husbandry and Veterinary Medicine, Southern Agriculture College, The Ministry of Agriculture and Rural Development. In 2010, he applied and got the scholarship “The Graduate Scholarship Programs for Neighboring Countries” from Chulalongkorn University, Thailand. He studied in the field of Swine Medicine at Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University during 2010-2012.