P28 Cloning and Expression of ORF4 and ORF6 Genes of Thai Porcine Reproductive and Respiratory Syndrome Isolate in *E. coli*

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**Introduction**

Porcine reproductive and respiratory syndrome virus (PRRSV) is characterized by respiratory problems in young pigs and severe reproductive failure in sows, including abortion, stillbirths, and weak piglets. PRRS viral genome has 8 encode open reading frames (ORF) of 1A, 1B, 2, 3, 4, 5, 6 and 7, respectively. Among these genes, the ORF4 gene has neutralizing epitope and can induce humoral immune responses (1) while the ORF6 gene also has highly antigenicity which induces antibody response about 10 days after infection. In this study, we first report the cloning of ORF4 and ORF6 genes of Thai PRRSV isolate and the expression of these gene products in *E. coli* bacterial system.

**Materials and Method**

A Thai PRRSV isolate classified as the US genotype obtained from field-sample were used for this study. The Viral RNA was extracted from bronchial alveolar lavage by a commercial kit (Qaigen, USA). The RT-PCR which amplified ORF4 and ORF6 were performed with the specific primers. The primer1 (5'ATG GCT TCG TCC CTT CTT TTC 3') and primer2 (5'TCA AAT TGA CAA CAG AAT GGC 3') were designed for the ORF4. The primer3 (5'ATG GGG TCG TCC TTA GAT GAC 3') and primer4 (5'TTA TTT GGC ATA TTT GAC AAG 3') was designed for the ORF6. The PCR products were purified, cloned into pCR®8/GW/TOPO® vector (Invitrogen, USA) and transformed into host cell *E. coli* strain DH5α. Then, the products were subcloned into the expression vector pGEX-4T2 which had GST fusion proteins and transformed into *E. coli* strain JM109 and used IPTG for induction of protein expression. The expressed recombinant GST-ORF4 and GST-ORF6 fusion proteins were analyzed by 10% SDS-PAGE and CBB staining.

**Results and Discussion**

The ORF4 and ORF6 genes were 537 and 525 bps; respectively (Fig. 1). Comparison of the amino acid sequences, ORF4 and ORF6 sequences were approximately 91% and 99.8% homologies with VR2332 (US strain) and O1NP1.2 (Thai isolate); respectively (data not shown). For cloning, ORF4 and ORF6 genes were successfully cloned into the pCR®8/GW/TOPO® vector and subcloned into the pGEX-4T2 vectors. The pGEX4T2-ORF4 and pGEX4T2-ORF6 were successfully expressed in *E. coli* strain JM109. The optimal conditions for the expression were induced with 100 mM IPTG for 3 hours at 37°C. The recombinant GST fusion-ORF4 and GST fusion-ORF6 proteins had approximated molecular mass of 45 kDa (GST 26 kD + ORF4 19 kD) and 44 kDa (GST 26 kD + ORF6 18 kD); respectively (Figs. 2 and 3). The purification and the antigenicity by using western blot with sera from infected PRRSV pigs of these recombinant fusion proteins will be further investigation.

**References**