

# RNA Interference Targeting Nucleocapsid Protein Inhibits Porcine Reproductive and Respiratory Syndrome Virus Replication in Marc-145 Cells

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Porcine reproductive and respiratory syndrome (PRRS) is an important disease, which leads to severe economic losses in swine-producing areas of the world. However, current antiviral strategies cannot provide highly effective protection. In this study, three theoretically effective interference target sites (71-91, 144-164, 218-238) targeting the nucleocapsid (N) gene of PRRSV were designed and selected, and then three siRNA-expressing plasmids were constructed, respectively named p2.1-N71, p2.1-N144, and p2.1-N218. The recombinant siRNA-expressing plasmids were transfected into Marc-145 cells; then the cells were infected with PRRSV (JL07SW strain); finally, after incubation for 48 h, the antiviral activity of those siRNA-expressing plasmids in Marc-145 cells was assessed by cytopathic effects, virus titers, indirect immunofluorescence, and quantitative real-time PCR. Experimental results demonstrated that these three siRNA-expressing plasmids could effectively and significantly inhibit the replication of PRRSV by 93.2%, 83.6%, and 89.2% in Marc-145 cells, respectively. Among these three siRNA-expressing plasmids, p2.1-N71 was found to be most effective, while p2.1-N144 and p2.1-N218 displayed relatively weak inhibition of virus replication. The results indicated that siRNA-expressing plasmids targeting the N gene of PRRSV could significantly inhibit PRRSV replication in Marc-145 cells. Based on our experimental results and previous reports, the 71-91, 179-197, and 234-252 sites of the N gene are good choices to effectively inhibit the replication of PRRSV, and this RNA interference technique can be a potential anti-PRRSV strategy.

**Keywords:** PRRSV, RNA interference, replication, nucleocapsid protein, Marc-145 cells

## Introduction

Porcine reproductive and respiratory syndrome (PRRS) is an important viral disease that was first described in North America in 1987 and subsequently in Europe in 1990 and has since become a problem to the swine industry worldwide (Wensvoort *et al.*, 1991; Sagong and Lee, 2011). It is characterized as respiratory disorders in young pigs and can cause reproductive failure in sows. The causative agent, PRRS virus (PRRSV), is a small, enveloped single-stranded, positive-sense RNA virus, which is classified as a member of the order of *Nidovirales*, family *Arteriviridae*, genus *Arterivirus* (Meulenber *et al.*, 1993; He *et al.*, 2007). PRRSV can be divided into two major genotypes: the North American strains (e.g., VR-2332), and the European strains (e.g., Lelystad virus, LV). The PRRSV genome with a size of approximately 15 kb in length comprises at least 9 open reading frames (ORF1a, ORF1b, ORF2a, ORF2b, ORF3-7) (Lee and Yoo, 2005). The ORF1a and ORF1b encode the non-structural proteins, replicase and polymerase, which are believed to be involved in viral replication (Brockmeier *et al.*, 2012). The ORFs 2-7 are postulated to encode for structural proteins (Cao *et al.*, 2010; Zhang *et al.*, 2013). ORFs 2-4 encode three membrane-associated minor glycoproteins (Sun *et al.*, 2012). ORF5 encodes the major envelope glycoprotein (GP5) in virions, ORF6 encodes M protein which is the unglycosylated membrane protein (Lee and Yoo, 2005; Lu *et al.*, 2012), and ORF7 encodes the nucleocapsid (N) protein which constitutes about 20–40% of the total protein content of the virion and is essential to the assembly and disassembly of the virion (Li and Ren, 2011). As previously described, the N protein was highly antigenic (Dea *et al.*, 2000), which therefore made it a suitable candidate for the detection of virus-specific antibodies and for diagnosis of the disease (Dea *et al.*, 2000; Bao *et al.*, 2012). Meanwhile, as originally reported, it may be the most immunogenic protein of the virus (Meulenber *et al.*, 1995), but recent findings disclosed that anti-N antibodies were non-neutralizing and non-protective (Murtaugh *et al.*, 2002; Terje, 2010). However, when compared to the other major structural proteins of PRRSV, the N protein displays a high degree of conservation.

RNA interference (RNAi) or RNA silencing is a revolution in biology (Siomi and Siomi, 2009). It is the process whereby double-stranded RNA (dsRNA) induces the sequence-specific degradation of homologous messenger RNA (mRNA) (Ding *et al.*, 2013). A long dsRNA which enters into the cell is cleaved to small interfering RNA (siRNA) duplexes (21–25 nucleotides in length) by a member of the Dicer family

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**Table 1. List of the siRNA target sequences.** For each target site, we designed complementary 55–60 mer oligonucleotides. For the forward 5' single-stranded oligonucleotides, the first five lowercase nucleotides represent a *Bam*HI site (gatcc) for ligation into the pSilencer vectors; the subsequent uppercase oligonucleotides represent the 19 nt sense and antisense sequences specific to the mRNA target; the italics section represents a 9 nt loop sequence common to all of the hairpins, and polythymidine tracts involved in the termination of transcription are underlined (TTTTTT).

Target sites	the siRNA target sequences
N-71 up	5'-gatccATGCTGGGTAAGATCATCG <b><i>TTCAAGAG</i></b> ACGATGATCTTACCCAGCATT <u>TTTTTT</u> GGAAA-3'
N-71 down	5'-agcttTTCCAAAAAATGCTGGGTAAGATCATCG <b><i>TCTCTTGA</i></b> ACGATGATCTTACCCAGCATG-3'
N-144 up	5'-gatccACCCGGAGAAGCCCCATTT <b><i>CTCAAGAG</i></b> AAAATGGGGCTTCTCCGGGT <u>TTTTTT</u> GGAAA-3'
N-144 down	5'-agcttTTCCAAAAAACCCTGGAGAAGCCCCATTT <b><i>TCTCTTGA</i></b> AAAATGGGGCTTCTCCGGGTG-3'
N-218 up	5'-gatccTTGTGTCTGTCGTCGATCC <b><i>TTCAAGAG</i></b> AGGATCGACGACAGACACA <u>TTTTTT</u> GGAAA-3'
N-218 down	5'-agcttTTCCAAAAAATTGTGTCTGTCGTCGATCC <b><i>TCTCTTGA</i></b> AGGATCGACGACAGACACAACG-3'
Control up	5'-gatccGAGGTCACGGGTCAGTTT <b><i>ATCAAGAG</i></b> ATAAAGTACCCGTGACCT <u>TTTTTT</u> GGAAA-3'
Control down	5'-agcttTTCCAAAAAAGAGGTCACGGGTCAGTTT <b><i>TCTCTTGA</i></b> ATAAAGTACCCGTGACCTCG-3'

of ribonuclease-III-like enzymes (Whangbo and Hunter, 2008). These duplexes are unwound and one strand is loaded into the RNA-induced silencing complex (RISC), which effectively searches the transcriptome and finds potential target RNAs. The loaded single-stranded RNA (ssRNA) directs an endonuclease to cleave mRNA that contains sequence homologous to the ssRNA. In this way, the guide strand determines the sequence specificity of the RNA interference response (Siomi and Siomi, 2009). So when RNA interference was first found, it was considered to be one of the most promising methods to combat viral infection (Ullu *et al.*, 2004). So far, RNA interference has been applied to combat several serious viral diseases, such as Newcastle disease (Yin *et al.*, 2010), influenza (Ge *et al.*, 2003), foot and mouth disease (Chen *et al.*, 2004), porcine transmissible gastroenteritis (Zhou *et al.*, 2007), classical swine fever (Porntrakulpipat *et al.*, 2010), porcine circovirus disease (Sun *et al.*, 2007), avian metapneumo virus (Ferreira *et al.*, 2007), and SARS (Wu *et al.*, 2005). Therefore, based on previous research on the application of RNAi technique on viral diseases, and because of the high conservation of the two major genotypes of the PRRSV N gene, we explored the application of the RNAi technique to inhibit the replication of PRRSV.

## Materials and Methods

### Virus and cells

The PRRSV field strain JL07SW, a highly pathogenic PRRSV belonging to the North American genotype, was isolated in 2007 by our research group from the lungs of pigs from an infected breeding farm in Jilin province (China) during a severe PRRSV outbreak as previously reported (Zhang *et al.*, 2012). Marc-145 cells was cultured in Dulbecco's modified

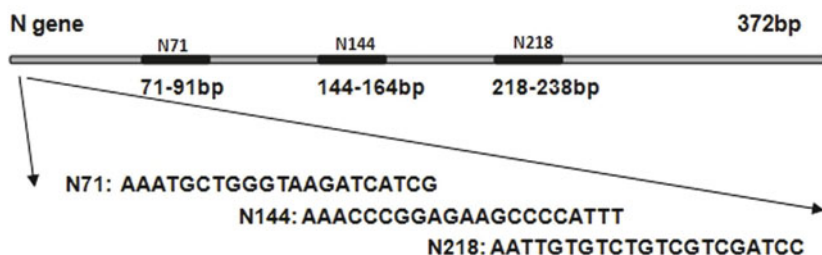
Eagle's medium (DMEM, Invitrogen, USA) supplemented with 8% heat-inactivated fetal bovine serum (FBS, GIBCO, USA) (pH 7.4), 2 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin (Invitrogen). The maintenance medium was supplemented with 2% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin.

### Selection of target sequences and construction of siRNA-expressing plasmids

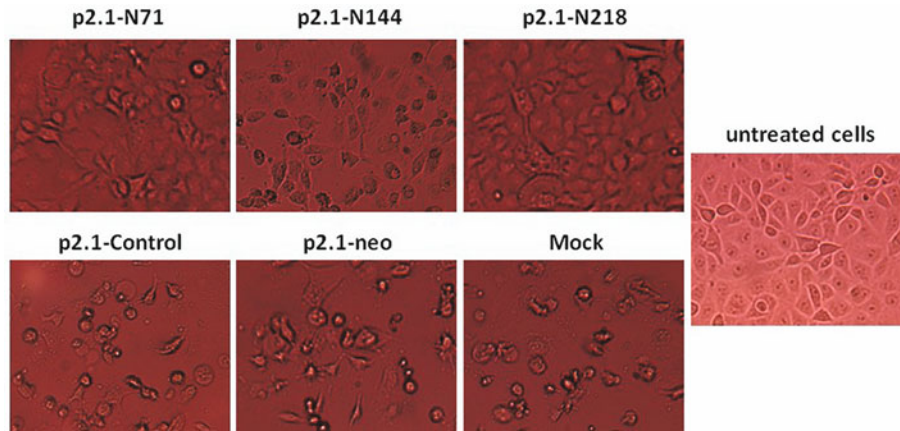
Utilizing siRNA Target finder tool ([http://www.ambion.com/techlib/misc/siRNA\\_finder](http://www.ambion.com/techlib/misc/siRNA_finder)), three of the best theoretical siRNA target sequences against the N gene of PRRSV (GenBank accession no. KF938947) (Fig. 1) and one scrambled negative control siRNA sequence were designed, selected (as shown in Table 1), and synthesized by Shanghai Sangon Biotech (China). siRNA duplexes were resuspended in DEPC-treated water to obtain 20 µM solutions, which was then heated at 90°C for 1 min and incubated at 37°C for 60 min. Then the duplexes were digested with *Bam*HI and *Hind*III, and inserted into the pSilencer™ 2.1-U6 neo vector (Ambion, USA). The constructed recombinant plasmids were verified by sequencing, and respectively named p2.1-N71, p2.1-N144, p2.1-N218, and p2.1-Control.

### Cell culture, transfection, and virus infection

Marc-145 cells were trypsinized and seeded in 6-well plates at  $2.0 \times 10^5$  cells/well one day before transfection. When the cells obtained 70–80% confluency, they were washed three times with DMEM without antibiotics and FBS. Then transfection complexes containing 2 µg of each plasmid (p2.1-N71, p2.1-N144, p2.1-N218, and p2.1-Control) in 100 µl Opti-MEM medium mixed with 8 µl FuGENE HD transfection



**Fig. 1.** Three 21nt siRNA target sequences from three different regions of porcine reproductive and respiratory syndrome virus (PRRSV) N gene: N71, N144, N218.



**Fig. 2.** Cytopathic effect (CPE) of Marc-145 cells at 48 h after porcine reproductive and respiratory syndrome virus (PRRSV) infection. They show that viral replication is notably inhibited by transfection with siRNA-expressing plasmids p2.1-N71, p2.1-N144 and p2.1-N218. Virus-produced CPE was observed by light microscopy (150 $\times$ ) 48 h after virus inoculation.

reagent (Roche, Switzerland) were added according to the manufacturer's instructions. Specificity of the inhibition was confirmed by transfecting the cultures with the empty pSilencer<sup>TM</sup> 2.1-U6 neo vector and the nonspecific plasmid p2.1-Control. The optimal density of the transfection reagent was determined at 2  $\mu$ g in preliminary experimentation. 8 h after transfection, the transfection complexes were completely removed, and the cells were inoculated with PRRSV field strain JL07SW at a multiplicity of infection (MOI) of 0.5. The cultures were then incubated in a 5% CO<sub>2</sub> humidified atmosphere at 37°C for 1 h and then the culture medium was replaced by fresh DMEM maintenance medium. The transfected Marc-145 cells were then incubated for 48 h in a 5% CO<sub>2</sub> humidified atmosphere at 37°C. Finally, the inhibition efficiency was determined by cytopathic effects (CPE) observation, indirect immunofluorescence, and quantitative real-time PCR analysis.

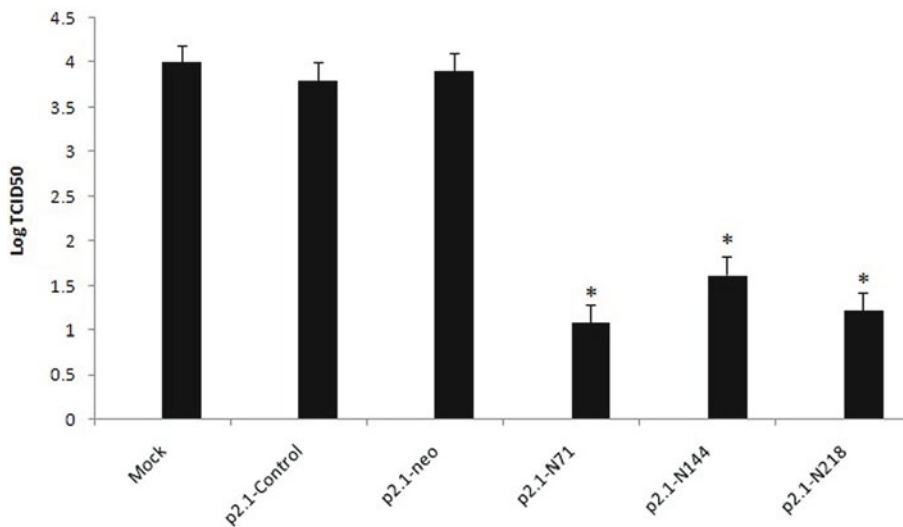
### Virus titration

Supernatant samples obtained from the above-described Marc-145 cells which were incubated at 37°C for 48 h after virus infection, were 10-fold serially diluted (from the lowest

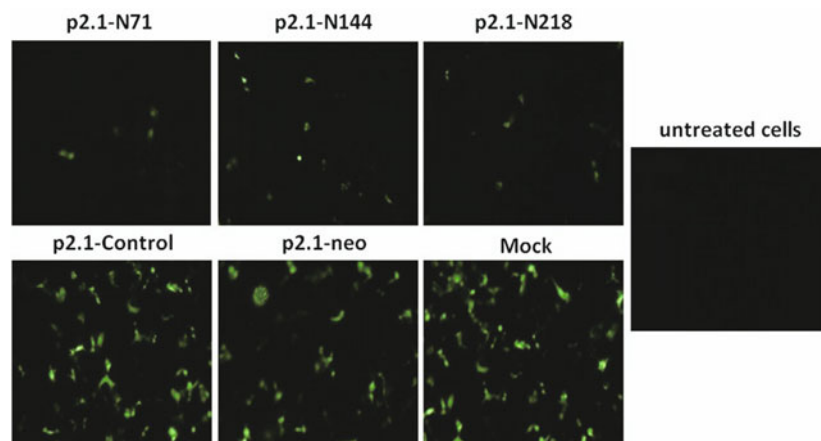
10<sup>-1</sup> to the highest 10<sup>-10</sup>), and each dilution was added to 96-well plates at a volume of 100  $\mu$ l/well. Each dilution was repeated in eight wells. Before this, Marc-145 cells had been cultured in the 96-well plates for 24 h. After incubating the 96-well plates for 3 days, TCID<sub>50</sub> (the 50% tissue culture infectious dose) was calculated by the method of Spearman-Kärber (Kärber *et al.*, 1931).

### Indirect immunofluorescence

48 h after virus infection, Marc-145 cells cultured in 6-well plates were fixed with cold methanol for 30 min on ice. Following three washes by phosphate-buffered saline (PBS, pH 7.4), the fixed cells were incubated with anti-PRRSV antibodies at a dilution of 1:100 for 1 h at 37°C. Unbound antibodies were washed three times with 5 min incubation with PBS containing 0.1% Tween. Then, the cells were incubated with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-pig IgG at a dilution of 1:1000 for 1 h in a 5% CO<sub>2</sub> humidified atmosphere at 37°C. Then the cells were washed three times again, and mounted with 90% glycerine and then analyzed by fluorescence microscopy.



**Fig. 3.** siRNA constructs interfere with porcine reproductive and respiratory syndrome virus (PRRSV) production in Marc-145 cells at 48 h post virus infection. Then cell supernatants were collected from these cells for viral titration. All data were obtained from experiments performed in triplicates. The titers on y-axis are shown as logarithmic value.



**Fig. 4.** Determination of Marc-145 cell monolayers transfected with or without siRNA-expressing plasmids targeting N gene of PRRSV by indirect immunofluorescence assay (IFA). Cells were visualized with a fluorescence microscope, Magnification (150 $\times$ ). The bright green color means that the expression of N protein in Marc-145 cells is not blocked. Black color means that the replication of PRRSV in Marc-145 cells has been inhibited.

### Quantitative real-time PCR analysis

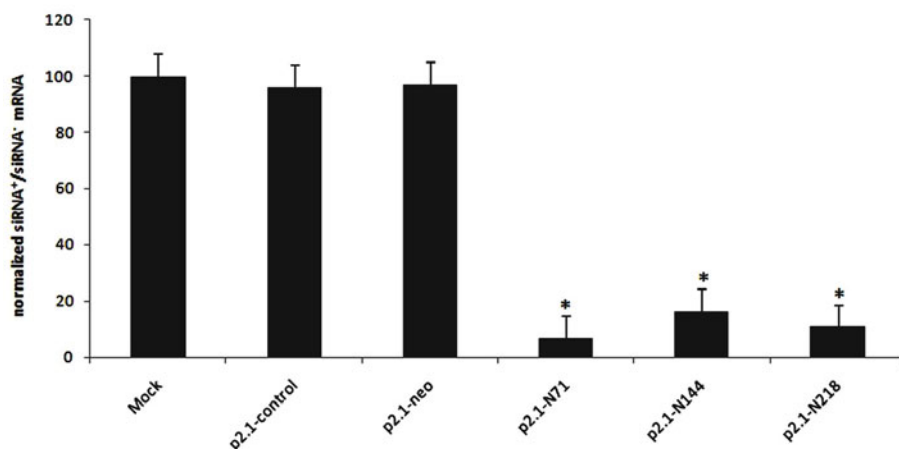
To investigate the effect of RNA interference on PRRSV replication, mRNA transcription level of N gene was evaluated by quantitative real-time PCR. 48 h after virus infection, the culture medium of the Marc-145 cells was removed and the cells were harvested. Total RNA was extracted from transfected and untransfected cells using Trizol Reagent (Invitrogen, USA) according to the manufacturer's instructions. To eliminate contamination of DNA, the samples were incubated with RNase-Free DNase Set (TaKaRa, Japan) for 30 min at 37 $^{\circ}$ C, and then DNase was inactivated by incubation at 65 $^{\circ}$ C for 10 min. Reverse transcription was carried out in a 20  $\mu$ l reaction volume at 42 $^{\circ}$ C for 1 h, containing 8  $\mu$ l of RNA extract, 0.5  $\mu$ l random primer, 4  $\mu$ l 5 $\times$ reaction buffer, 2  $\mu$ l 2.5 mmol/L dNTP, 0.6  $\mu$ l Ribonuclease Inhibitor (BBI, USA), and 0.5  $\mu$ l reversed transcriptase enzyme M-MLV (MBI, USA). To evaluate the mRNA transcription of N and  $\beta$ -actin, quantitative real-time PCR using RNA-direct<sup>TM</sup> SYBR Green Real-time PCR Master Mix (Toyobo Bio Co. Ltd., Japan) was performed. For the quantitative real-time PCR, we designed the N gene specific primers to amplify the 99–277 sites (179 bp) of the N gene. The primers for the N gene and  $\beta$ -actin are: 1) N-F, 5'-AACCAGTCCAgAggCAA gg-3'; N-R, 5'-gACAgggCACAAGTTCCAg-3' 2)  $\beta$ -actin-F, 5'-TGACTGACTACCTCATGAAGATCC-3';  $\beta$ -actin-R, 5'-

TCTCCTTAATGTCACGCACGATT-3' (Xiao *et al.*, 2011). All reactions were done in a 50  $\mu$ l reaction volume, containing 2 U ExTaq polymerase, 5  $\mu$ l 10 $\times$ buffer, 1.5 mM MgCl<sub>2</sub>, 0.5  $\mu$ M primers (synthesized by TaKaRa, China), 200  $\mu$ M deoxynucleoside triphosphates, and the 2  $\mu$ l product of reverse transcription reaction mixture (used as a template). An initial denaturation step of 5 min at 95 $^{\circ}$ C was followed by 45 cycles of 45 sec at 94 $^{\circ}$ C, 45 sec at 55 $^{\circ}$ C, and 45 sec at 72 $^{\circ}$ C, with a final extension step of 8 min at 72 $^{\circ}$ C. Amplification and detection of samples were performed with the Rotor-gene 3000 detection system (Bio-Rad, DNAEngine). The standard curves were plotted by linear extrapolation of the Ct value.

## Results

### Detection of cytopathic effect

CPEs were observed daily during the incubation period. 48 h post infection, as shown in Fig. 2, the cells transfected with siRNA-expressing plasmids clearly showed less CPE. Moreover, p2.1-N71-treated cells showed better inhibition efficiency on PRRSV replication than p2.1-N144 or p2.1-N218-treated cells. The cells treated with the empty vector (p2.1-neo vector) and the cells treated with the p2.1-Control plasmid



**Fig. 5.** Quantitative real-time PCR results indicate a remarkable decrease in relative mRNA level of N gene in Marc-145 cells transfected with the p2.1-N71, p2.1-N144, and p2.1-N218 plasmids at 48 h after virus infection. The mRNA of  $\beta$ -actin served as an internal reference. The values shown are means of three independent experiments.

demonstrated the same typical PRRSV-induced CPE as the Mock cells (untransfected but PRRS virus-infected Marc-145 cells). CPE mainly contained pycnotic, the accumulation of detached cells, and detached from the monolayer.

### Virus titration

To measure the inhibition efficiency of siRNAs on viral replication in Marc-145 cells, the supernatants of the cells infected with PRRSV for 48 h were obtained and used for virus titration through TCID<sub>50</sub>. As shown in Fig. 3, the virus titer of Marc-145 cells transfected with p2.1-N71, p2.1-N144, and p2.1-N218 (874-fold, 151-fold, and 592-fold reduction, respectively) were significantly lower than the virus titer of the cells transfected with p2.1-Control or p2.1-neo ( $P < 0.05$ ). In addition, the cells transfected with p2.1-N71 showed the best inhibition efficiency.

### Indirect immunofluorescence assay

In order to confirm the effect of siRNA-expressing plasmids in inhibiting the replication of PRRSV, indirect immunofluorescence assay was also performed and observed under a fluorescence microscope. The results indicated that fewer fluorescing cells were seen in the monolayers transfected with the siRNA-expressing plasmids (p2.1-N71, p2.1-N144, and p2.1-N218). Interestingly, cells transfected with p2.1-N71 in the monolayer had less fluorescein than the other two siRNA-transfected monolayers. In contrast, when compared to the mock cells, the cells transfected with p2.1-control and p2.1-neo had equivalent obvious fluorescein (as shown in Fig. 4).

### Quantitative real-time PCR analysis

To investigate whether siRNAs could effectively inhibit the replication of PRRSV in Marc-145 cells, the mRNA level of transcribed N gene was evaluated by quantitative real-time PCR. The correlation coefficients ( $R^2$ ) of the standard curves were 0.999 ( $\beta$ -actin) and 0.991 (N gene), respectively. All PCR amplification efficiencies ( $E$ ) were  $>0.96$  with only one peak in all dissociation curves. Transcribed levels of PRRSV N gene were normalized to  $\beta$ -actin using the  $2^{-\Delta\Delta CT}$  method. As shown in Fig. 5, compared to the mock group at 48 h post-infection, the results indicated that the mRNA levels of transcribed PRRSV N gene were remarkably reduced by 93.2%, 83.6%, and 89.2% in the Marc-145 cells respectively transfected with p2.1-N71, p2.1-N144, and p2.1-N218 ( $P < 0.05$ ). Moreover, the cells transfected with p2.1-N71-expressing plasmid had the lowest mRNA transcription level of the N gene. These results demonstrated that the constructed siRNA-expressing plasmids could specifically and effectively inhibit the expression of the PRRSV N gene, and thus suppress PRRSV replication.

### Discussion

RNAi was first discovered in *Trypanosoma brucei*, a species of the family *Trypanosomatidae*, and it rapidly became the method of choice to downregulate gene expression in the organisms (Fire *et al.*, 1998; Ullu *et al.*, 2004). For example,

Ge *et al.* (2003) has demonstrated that siRNA specific for nucleocapsid or a component of the RNA transcriptase could potentially inhibit influenza virus production in both cell lines and embryonated chicken eggs. Chen *et al.* (2004) has shown that the transfection of siRNA-expressing plasmids gave an 80% to 90% reduction in the expression of foot-and-mouth disease virus VP1 gene in BHK-21 cells. Zhou *et al.* (2007) constructed two siRNA-expressing plasmids targeting the RNA-dependent RNA polymerase gene of porcine transmissible gastroenteritis virus, and experimental results demonstrated that the amounts of viral RNAs in cell cultures pre-transfected with the two plasmids were reduced by 95.2% and 100%, respectively. Porntrakulpipat *et al.* (2010) has demonstrated that siRNA targeting the nucleocapsid protein of the classical swine fever virus (CSFV) effectively inhibited CSFV replication. Therefore, siRNA can play an important role in the inhibition of viral replication. Likewise, siRNA technique is also applied to fight against PRRSV. Guo *et al.* (2013) has shown that delivered microRNA-181 mimics could strongly inhibit PRRSV replication *in vitro* through specifically binding to a highly conserved region (over 96%) in the downstream of GP4 of the viral genomic RNA, but the inhibition of PRRSV replication was dose dependent. Moreover, Bao *et al.* (2012) has constructed four siRNAs targeting ORF1b and ORF6, and verified that RNAi could effectively inhibit PRRSV replication in cultured cells *in vitro*.

The N gene is directly involved in the virus replication. As previously described in a report, the N gene could affect PRRSV function involved in the recognition of viral RNA, sub-cellular localization and interactions with other viral and cellular proteins (Stefanie *et al.*, 2012), and virus assembly. Therefore, the N gene plays a vital role during the life cycle of PRRSV. Based on these characteristics of the N protein, this study utilizes the RNAi technique to target the N gene to inhibit the replication of PRRSV. We selected the N gene as a target gene, and then three siRNAs were designed at 71-91, 144-164, 218-238 sites (as shown in Fig. 1) for RNA interference. Through the assessments of CPE observation, virus titers, indirect immunofluorescence and quantitative real-time PCR, the experimental results showed that these three siRNA plasmids could effectively inhibit the replication of PRRSV. They were able to prevent Marc-145 cells from forming CPE, and could make 874-fold, 151-fold, and 592-fold decreases of virus titers detected by TCID<sub>50</sub>. Furthermore, the results of quantitative real-time PCR showed that three siRNA-expressing plasmids could effectively inhibit the replication of PRRSV by 93.2%, 83.6%, and 89.2% respectively in Marc-145 cells. Among these three siRNA-expressing plasmids, p2.1-N71 was found to be most effective, while p2.1-N144 and p2.1-N218 displayed relatively weak inhibition of virus replication. A potential explanation for the most effective inhibition by p2.1-N71 is that an A/U rich region plays a crucial role for the antisense terminal duplex regions of highly effective siRNAs. The A/U rich region at the 5' antisense end of the sequence may be required for target recognition and contribute to the unwinding of siRNAs (Ui-Tei *et al.*, 2004). According to this guideline, after analysis on the three siRNA sequences (p2.1-N71, p2.1-N144, and p2.1-N218), we find out that p2.1-N71 has more A/U at the 5' antisense end than p2.1-N144 and p2.1-N218

(namely, siRNA sequences of p2.1-N71 in A/U rich terminal region is up to 53%, while p2.1-N144 and p2.1-N218 are 42% and 47% respectively). Therefore, p2.1-N71 shows the most effective inhibition rather than p2.1-N144 or p2.1-N218. Similar reports have been seen in He's (He *et al.*, 2007) and Huang's studies (Huang *et al.*, 2006). He *et al.* (2007) constructed four U6 promoter-encoded siRNA sequences directing against a well-conserved region of PRRSV N gene. Among these four targets, N179 was found to be highly effective, with an inhibition rate of 96%, while N95, N218, and N294 displayed weak activity (the inhibition rates were 49%, 42%, and 51%, respectively). Huang *et al.* (2006) constructed four siRNA-expressing plasmids targeting the coding region of the N protein of PRRSV. The experimental results demonstrated that the siRNA could effectively induce a potent repression of gene expression in Marc-145 cells, and cells transfected with plasmids pSUPER-N3 siRNA targeting the 234-252 sites of the N gene showed a significant decrease in virus yield when compared to the 13-31, 100-118, and 294-312 sites of the N gene. Coincidentally, N179 and pSUPER-N3 both basically meet the A/U rich guideline.

Additionally, blasting the N gene of PRRSV JL07SW strain in NCBI revealed that there was 100% nucleotide sequence homology with the N gene of at least 32 different PRRSV strains which belong to Northern American type PRRSV. In theory, these siRNAs should inhibit the replication of those 32 Northern American strains of PRRSV, but owing to the shortage of PRRSV strains in our labs, we could only use the JL07SW strain of PRRSV for the experiments. Furthermore, there was only 59.6% nucleotide sequence homology with the LV strain, Mik strain and Sel strain (typical European strains). Therefore, we deduce that N71 is a good target to suppress Northern American PRRSV replication by RNAi technique.

In conclusion, based on our experimental data and the results in the literatures, the 71-91, 179-197, and 234-252 sites of the N gene of PRRSV would be good choices to design siRNA to inhibit the replication of PRRSV, and this RNAi technique could be a potential candidate method for the treatment of PRRSV. Of course, further study is required to determine whether such treatment could fight against PRRSV replication and propagation *in vivo*.

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