**Open reading frames 1a and 1b of the porcine reproductive and respiratory
syndrome virus (PRRSV) collaboratively initiate viral minus-strand RNA
synthesis**

Yan-Dong Tang^{1§}, Qiong-Qiong Fang^{1§}, Ji-Ting Liu^{1,2}, Tong-Yun Wang¹, Yu Wang¹,
Ye Tao¹, Yong-gang Liu^{1*}, Xue-Hui Cai^{1*}

1, The Key Laboratory of Veterinary Public Health, Ministry of Agriculture, State Key
Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute of the
Chinese Academy of Agricultural Sciences, Harbin 150001, China

2, College of Animal Science and Technology, Jilin Agriculture University,
Changchun 130018, China

Footnotes:

§ These authors contributed equally to this article.

*Corresponding author:

Xue-Hui Cai: Harbin Veterinary Research Institute of the Chinese Academy of
Agricultural Sciences, Harbin 150001, China. Tel.: +86-13504512466. E-mail:
cai139@hvri.ac.cn.

Yong-gang Liu: Harbin Veterinary Research Institute of the Chinese Academy of
Agricultural Sciences, Harbin 150001, China. Tel.: +86-18686861601. E-mail:
yonggangliu@aliyun.com.

Abstract

The porcine reproductive and respiratory syndrome virus (PRRSV) causes a persistent threat to the swine industry, especially when highly pathogenic PRRSV (HP-PRRSV) emerges. Previous studies have indicated that PRRSV RNA synthesis was correlated with HP-PRRSV virulence. PRRSV RNA synthesis includes genomic RNA and sub-genomic mRNA, and these processes require minus-strand RNA as a template. However, the mechanisms involved in PRRSV minus-strand RNA synthesis are not fully understood. A mini-genome system can be used to assess viral replication mechanisms and to evaluate the effects of potential antiviral drugs on viral replicase activities. In this study, we developed a mini-genome system that uses firefly luciferase as a reporter. Based on this system, we found that PRRSV RNA-dependent RNA polymerase nsp9 alone failed to activate virus minus-strand RNA synthesis. We also demonstrated that combinations of open reading frames 1a (ORF1a) and ORF1b are necessary for viral minus-strand RNA synthesis.

Keywords: PRRSV, Mini-genome system, RNA synthesis

1. Introduction

The porcine reproductive and respiratory syndrome virus (PRRSV) is a positive-strand RNA virus that is approximately 15 kb in length and belongs to the Arteriviridae family [1]. PRRSV causes substantial economic losses to the global swine industry, especially with the emergence of highly pathogenic PRRSV (HP-PRRSV). Most recently, there have been devastating outbreaks of recombinant PRRSV [2,3]. The PRRSV genome includes open reading frame 1a (ORF1a), ORF1b and ORFs 2~7 [1]. ORFs 2~7 mainly encode viral structural proteins and have been extensively studied [1,4,5]. ORF1a and ORF1b encode polyproteins, which are further processed into smaller protein products, designated as nonstructural proteins (nsps). Based on bioinformatics predictions and experimental studies, 12 nsps have been identified in PRRSV [6].

Viral RNA synthesis plays an important role in the PRRSV life cycle. The key enzymes for PRRSV RNA synthesis are encoded in ORF1b, specifically the viral RNA-dependent RNA polymerase (RdRp, nsp9), which is essential for genome replication and synthesis [6]. Nsp10 is an RNA helicase, which can unwind dsRNA [7]. Both nsp9 and nsp10 are the key enzymes for arterivirus RNA synthesis, and they have been reported as responsible for the virulence of HP-PRRSV [6,8]. However, little is known about this process. PRRSV can only replicate in limited cell lines, and these permissive cell lines always feature low transfection efficacy. Therefore, studies on the mechanisms of PRRSV RNA synthesis in these cells are difficult.

Mini-genome systems have been used to study viral replication mechanisms and to evaluate the effects of antiviral drugs. For many RNA viruses, mini-genome systems have been used to evaluate viral polymerase activity and to perform high-throughput screens of virus inhibitors, such as studies evaluating influenza virus, Marburg virus and Ebola virus [9,10,11,12,13,14]. Generally, mini-genome systems possess a reporter gene that is used for evaluating the RNA synthesis level, but they lack the corresponding RNA synthesis polymerase. Furthermore, these systems can easily work in transfected cells. As a consequence, mini-genome systems are particularly suited for studying aspects of viral RNA synthesis. For this purpose, we

developed a PRRSV-derived mini-genome system to analyze RNA synthesis in our study.

2. Materials and Methods

2.1 Cells and virus stocks

Marc145 cells and HEK293 cells were maintained in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (FBS). The PRRSV HuN4 strain is an HP-PRRSV strain that was isolated from a farm in 2006 (GenBank accession no. EF635006) [15]. The infectious PRRSV titer (50% tissue culture infection dose, TCID₅₀) was determined on Marc145 cells.

2.2 Plasmids

HuN4 infectious cDNA clones were constructed as described in Figure 1A of our previous study [16]. For PRRSV mini-genome system construction, we first individually amplified PCR products of three key mini-genome system elements. CMV-F and CMV-R primers were used to amplify the fragment from CMV to the 5' untranslated region (UTR) of the infectious clone. Luc-F and Luc-R primers were used to amplify the firefly luciferase reporter gene from pGL3Basic (Clontech, USA). BGH-F and BGH-R were used to amplify the fragment from the 3'UTR to BGH poly (A) signals. Mini-genome systems were constructed with overlapping PCR using the abovementioned primers and were then cloned into Tang-EGFP vectors [17]. The nsp9 plasmid was created using nsp9-F and nsp9-R primers. The nsp10 plasmid was generated using nsp10-F and nsp10-R primers. ORF1a and ORF1b were constructed using the ORF1a forward and reverse primers and the ORF1b forward and reverse primers, respectively. These genes were cloned from HuN4 infectious cDNA and expressed using the expression vector pCMV-HA (Clontech, USA), and they were fused to an N-terminal HA tag. All constructs were confirmed by DNA sequencing. The sequences of the PCR primers are summarized in Table 1.

2.3 Transfection and luciferase activity determination

Marc145 cells were seeded in 48-well plates. At approximately 90% confluence, 0.2 µg of the mini-genome system plasmid DNA and 0.01 µg of Renilla luciferase plasmid (Promega, USA) were transfected into cells using the X-tremeGENE HP DNA transfection reagent (Roche, Switzerland) following the manufacturer's protocol. The Renilla luciferase activity was used as an internal control for the normalization of luciferase values obtained from cells transfected with the firefly luciferase mini-genome system. At 18 h post-transfection, each well with Marc145 cells was infected with 0.1 multiplicity of infection (MOI) of PRRSV HuN4. Twenty-four hours later, the cells were lysed and assayed for firefly luciferase activity using a dual-luciferase reporter assay system (Promega, USA) according to the manufacturer's instructions.

HEK293 cells were seeded in 48-well plates. At approximately 90% confluence, 0.2 µg of the mini-genome system plasmid DNA and 0.01 µg of Renilla luciferase plasmid were co-transfected with the indicated plasmids (1 µg) using the X-tremeGENE HP DNA transfection reagent. At 48 h post-transfection, firefly luciferase activity was measured as described above.

2.4 Western blots

PRRSV-infected Marc145 cells were washed with cold PBS three times and then lysed in radioimmunoprecipitation assay (RIPA) lysis buffer containing a protease inhibitor cocktail (Roche, Switzerland). The proteins in the cell lysates were separated by SDS-PAGE, transferred to PVDF membranes (Millipore, USA), and probed with the indicated antibodies for detection.

3. Results and Discussion

3.1 PRRSV mini-genome system construction

The mechanisms of PRRSV minus-strand RNA synthesis are still unclear. The lack of an appropriate evaluation system for PRRSV minus-strand RNA synthesis

impedes such studies, and the mini-genome system described herein was designed to achieve this purpose. Mini-genome systems are powerful tools for understanding RNA synthesis mechanisms [9,10,11,12,13,14]. In our previous studies, a full-length cDNA clone of the HP-PRRSV strain HuN4 was cloned into the bacterial artificial chromosome (BAC) system (Fig. 1A) [16]. PRRSV-positive RNA transcription was driven by a CMV promoter. At the 3' end, the core sequence of hepatitis delta virus (HDV) ribozymes and a BGH poly (A) signal were placed downstream of the viral poly (A) tract. HDV ribozymes were used to ensure that the final transcripts had accurate terminations. The overall strategy to construct the mini-genome system is outlined in Figure 1B. The strategy involved using a large deletion for the removal of most of the gene coding regions and substituting these deletions with a reversed complement firefly luciferase gene. This designed mini-genome system can be used to evaluate the mechanism of PRRSV minus-strand RNA synthesis. pCMV initiates positive-strand RNA transcription, and if RdRp or RdRp complexes are present, then negative-sense luciferase RNA can be transcribed to mRNA for the synthesis of the luciferase reporter. Furthermore, to determine which element is essential for PRRSV minus-strand RNA synthesis, we constructed several support plasmids, as shown in Figure 1C.

3.2 The PRRSV mini-genome system works in PRRSV-infected cells

Because we know little about which element is critical for PRRSV minus-strand RNA synthesis, we first used live infectious PRRSV to evaluate this system. We transfected Marc-145 cells with a mini-genome system plasmid. After 18 h, 0.1 MOI

of PRRSV was inoculated. At 24 h post-infection, Firefly luciferase activity was normalized with respect to a co-transfected plasmid encoding Renilla luciferase. Similar results were obtained in three independent experiments. The results showed that the level of Firefly luciferase activity in the PRRSV-infected group was significantly higher than that in the control group (Fig. 2A). We confirmed the effective infection of PRRSV by Western blotting (Fig. 2B). This result indicated that our mini-genome system could be used as a tool to identify components for minus-strand RNA synthesis.

3.3 nsp9 and nsp10 failed to initiate minus-strand RNA synthesis

ORF1b is the most conserved portion of the arterivirus genome; it encodes four nsps, from nsp9 to nsp12. Nsp9 and nsp10 are the key enzymes for RNA-templated RNA synthesis; nsp9 is an RdRp, and nsp10 has ATPase activity and can unwind both RNA and DNA duplexes in a 5' to 3' direction [7,18]. Nsp9 and nsp10 have been reported to contribute to the fatal virulence of HP-PRRSV [8]. Additionally, HP-PRRSV was remarkably attenuated by the de-optimization of nsp9 codon pair bias [19]. All of these reports indicated that the correlation of nsp9 with virulence might be due to the influence of nsp9 on virus RNA synthesis. Therefore, in this study, we first tested whether nsp9 or nsp10 could initiate minus-strand RNA synthesis. Nsp9 and nsp10 were constructed as shown in Figure 1C. To our surprise, nsp9 or nsp10 co-transfected with the mini-genome system failed to activate the mini-genome system (Fig. 3A). Next, we speculated that nsp9 and nsp10 together might drive minus-strand RNA synthesis. However, a combination of nsp9 and nsp10 also failed

to activate the mini-genome system (Fig. 3B). This finding indicated that PRRSV minus-strand RNA synthesis might require other factors. Similar to how purified EAV nsp9 failed to utilize the 3' end of the viral genome as a template, this result suggested that additional factors are required [20].

3.4 Combinations of ORF1a and ORF1b are essential for minus-strand RNA synthesis

We further constructed the ORF1a and ORF1b support plasmids as shown in Figure 1C. However, ORF1a or ORF1b alone failed to activate the mini-genome system (Fig. 4A). Of note, when we combined ORF1a and ORF1b, the mini-genome system could be effectively activated as an infectious PRRSV infection (Fig. 4B). This finding indicated that both ORF1a and ORF1b were essential for PRRSV minus-strand RNA synthesis. It has been reported that both viral replication and the transcription complex (RTC) are associated with double-membrane vesicular structures (DMVs) in infected cells [21]. DMV formation is a typical feature for the life cycles of all arteriviruses [6,22], and this structure may provide a scaffold for viral RNA synthesis. PRRSV nsp2 and nsp3 are involved in DMV induction [23,24]. In addition, nsp1 functions relate to the regulation of subgenomic mRNA synthesis [25]. Furthermore, nsp7 has been shown to play a critical role in PRRSV RNA synthesis [26]. Together, these findings indicated that successful PRRSV RNA synthesis requires several factors working together, which might be the reason why only transfected ORF1a and ORF1b together could initiate viral minus strand RNA synthesis. Our mini-genome system will be a valuable tool to investigate the replication mechanisms of PRRSV.

In summary, ORF1a and ORF1b of PRRSV collaboratively initiate viral minus-strand RNA syntheses. The mini-genome system described here can be a useful tool for future investigations of various aspects of PRRSV replication. In particular, further trans-complementation analysis will enable a better understanding of the role of specific nsps in the process of PRRSV minus-strand RNA synthesis. This mini-genome system can also be used to evaluate the effects of potential antiviral drugs on viral replicase activities.

Acknowledgments: This study was supported by grants from the National Natural Science Foundation of China (Grant No. 31302065) and the National Science Foundation of Heilongjiang [Grant No. ZD2015006]

References

- [1] J.J. Meulenbergh, PRRSV, the virus, *Vet Res* 31 (2000) 11-21.
- [2] L. Zhou, Z. Wang, Y. Ding, X. Ge, X. Guo, H. Yang, NADC30-like Strain of Porcine Reproductive and Respiratory Syndrome Virus, China, *Emerg Infect Dis* 21 (2015) 2256-2257.
- [3] K. Zhao, C. Ye, X.B. Chang, C.G. Jiang, S.J. Wang, X.H. Cai, G.Z. Tong, Z.J. Tian, M. Shi, T.Q. An, Importation and Recombination Are Responsible for the Latest Emergence of Highly Pathogenic Porcine Reproductive and Respiratory Syndrome Virus in China, *J Virol* 89 (2015) 10712-10716.
- [4] S. Dea, C.A. Gagnon, H. Mardassi, B. Pirzadeh, D. Rogan, Current knowledge on the structural proteins of porcine reproductive and respiratory syndrome (PRRS) virus: comparison of the North American and European isolates, *Arch Virol* 145 (2000) 659-688.
- [5] M.P. Murtaugh, M.R. Elam, L.T. Kakach, Comparison of the structural protein coding sequences of the VR-2332 and Lelystad virus strains of the PRRS virus, *Arch Virol* 140 (1995) 1451-1460.
- [6] Y. Fang, E.J. Snijder, The PRRSV replicase: exploring the multifunctionality of an intriguing set of nonstructural proteins, *Virus Res* 154 (2010) 61-76.
- [7] E.M. Bautista, K.S. Faaberg, D. Mickelson, E.D. McGruder, Functional properties of the predicted helicase of porcine reproductive and respiratory syndrome virus, *Virology* 298 (2002) 258-270.
- [8] Y. Li, L. Zhou, J. Zhang, X. Ge, R. Zhou, H. Zheng, G. Geng, X. Guo, H. Yang, Nsp9 and Nsp10 contribute to the fatal virulence of highly pathogenic porcine reproductive and respiratory syndrome virus emerging in China, *PLoS Pathog* 10 (2014) e1004216.

- [9] M. Liu, M.K. Lam, Q. Zhang, R. Elderfield, W.S. Barclay, P.C. Shaw, The Functional Study of the N-Terminal Region of Influenza B Virus Nucleoprotein, *PLoS One* 10 (2015) e0137802.
- [10] B. Zhou, J. Ma, Q. Liu, B. Bawa, W. Wang, R.S. Shabman, M. Duff, J. Lee, Y. Lang, N. Cao, A. Nagy, X. Lin, T.B. Stockwell, J.A. Richt, D.E. Wentworth, W. Ma, Characterization of uncultivable bat influenza virus using a replicative synthetic virus, *PLoS Pathog* 10 (2014) e1004420.
- [11] K. Yamada, H. Koyama, K. Hagiwara, A. Ueda, Y. Sasaki, S.N. Kanesashi, R. Ueno, H.K. Nakamura, K. Kuwata, K. Shimizu, M. Suzuki, Y. Aida, Identification of a novel compound with antiviral activity against influenza A virus depending on PA subunit of viral RNA polymerase, *Microbes Infect* 14 (2012) 740-747.
- [12] M. Fukuoka, M. Minakuchi, A. Kawaguchi, K. Nagata, Y.O. Kamatari, K. Kuwata, Structure-based discovery of anti-influenza virus A compounds among medicines, *Biochim Biophys Acta* 1820 (2012) 90-95.
- [13] C.Y. Su, T.J. Cheng, M.I. Lin, S.Y. Wang, W.I. Huang, S.Y. Lin-Chu, Y.H. Chen, C.Y. Wu, M.M. Lai, W.C. Cheng, Y.T. Wu, M.D. Tsai, Y.S. Cheng, C.H. Wong, High-throughput identification of compounds targeting influenza RNA-dependent RNA polymerase activity, *Proc Natl Acad Sci U S A* 107 (2010) 19151-19156.
- [14] L.S. Uebelhoer, C.G. Albarino, L.K. McMullan, A.K. Chakrabarti, J.P. Vincent, S.T. Nichol, J.S. Towner, High-throughput, luciferase-based reverse genetics systems for identifying inhibitors of Marburg and Ebola viruses, *Antiviral Res* 106 (2014) 86-94.
- [15] G.Z. Tong, Y.J. Zhou, X.F. Hao, Z.J. Tian, T.Q. An, H.J. Qiu, Highly pathogenic porcine reproductive and respiratory syndrome, China, *Emerg Infect Dis* 13 (2007) 1434-1436.
- [16] Y. Tao, Construction and pathogenicity evaluation of HP-PRRSV infectious clone and chimeric viruses. (In Chinese), Chinese Academy of Agricultural Sciences (2014).
- [17] Y.D. Tang, J.T. Liu, Q.Q. Fang, T.Y. Wang, M.X. Sun, T.Q. An, Z.J. Tian, X.H. Cai, Recombinant Pseudorabies Virus (PRV) expressing firefly luciferase effectively screened for CRISPR/Cas9 single guide RNAs and antiviral compounds, *Viruses* 8 (2016) 90.
- [18] A. Seybert, L.C. van Dinten, E.J. Snijder, J. Ziebuhr, Biochemical characterization of the equine arteritis virus helicase suggests a close functional relationship between arterivirus and coronavirus helicases, *J Virol* 74 (2000) 9586-9593.
- [19] L. Gao, L. Wang, C. Huang, L. Yang, X.K. Guo, Z. Yu, Y. Liu, P. Yang, W.H. Feng, HP-PRRSV is attenuated by de-optimization of codon pair bias in its RNA-dependent RNA polymerase nsp9 gene, *Virology* 485 (2015) 135-144.
- [20] N. Beerens, B. Selisko, S. Ricagno, I. Imbert, L. van der Zanden, E.J. Snijder, B. Canard, De novo initiation of RNA synthesis by the arterivirus RNA-dependent RNA polymerase, *J Virol* 81 (2007) 8384-8395.
- [21] K.W. Pedersen, Y. van der Meer, N. Roos, E.J. Snijder, Open reading frame 1a-encoded subunits of the arterivirus replicase induce endoplasmic reticulum-derived double-membrane vesicles which carry the viral replication complex, *J Virol* 73 (1999) 2016-2026.
- [22] J.M. Pol, F. Wagenaar, J.E. Reus, Comparative morphogenesis of three PRRS virus strains, *Vet Microbiol* 55 (1997) 203-208.
- [23] C.C. Posthuma, K.W. Pedersen, Z. Lu, R.G. Joosten, N. Roos, J.C. Zevenhoven-Dobbe, E.J. Snijder, Formation of the arterivirus replication/transcription complex: a key role for nonstructural protein 3 in the remodeling of intracellular membranes, *J Virol* 82 (2008)

4480-4491.

- [24] E.J. Snijder, H. van Tol, N. Roos, K.W. Pedersen, Non-structural proteins 2 and 3 interact to modify host cell membranes during the formation of the arterivirus replication complex, *J Gen Virol* 82 (2001) 985-994.
- [25] M.V. Kroese, J.C. Zevenhoven-Dobbe, J.N. Bos-de Ruijter, B.P. Peeters, J.J. Meulenberg, L.A. Cornelissen, E.J. Snijder, The nsp1alpha and nsp1 papain-like autoproteases are essential for porcine reproductive and respiratory syndrome virus RNA synthesis, *J Gen Virol* 89 (2008) 494-499.
- [26] M. Zhang, Z. Cao, J. Xie, W. Zhu, P. Zhou, H. Gu, L. Sun, S. Su, G. Zhang, Mutagenesis analysis of porcine reproductive and respiratory syndrome virus nonstructural protein 7, *Virus Genes* 47 (2013) 467-477.

Figure legends

Figure 1. Schematic illustration of mini-genome development. (A) Schematic of the infectious clone HP-PRRSV HuN4 strain. (B) Structure of the PRRSV mini-genome system. The reverse complement Luc reporter gene was cloned in genome (viral) sense between the 5' and 3' UTRs of PRRSV. (C) Schematic of viral support protein plasmids, indicating the genes that were sub-cloned into the pCMV-HA vector. Viral support protein plasmids used to drive the Luc mini-genome system are shown.

Figure 2. PRRSV infection successfully drives the mini-genome system in PRRSV-infected cells. (A) Marc145 cells were pre-transfected with 0.2 µg of the mini-genome system plasmid DNA and 0.01 µg of the Renilla luciferase plasmid. At 18 h post-transfection, each well was infected with 0.1 MOI of PRRSV HuN4. After 24 h, the cells were lysed and assayed for firefly luciferase, and (B) N protein expression was evaluated by Western blotting (representative image).

Figure 3. A combination of nsp9 and nsp10 failed to initiate viral minus-strand RNA synthesis. (A) Nsp9 or nsp10 failed to stimulate the mini-genome system. 0.2 µg of mini-genome system plasmid DNA and 0.01 µg of Renilla luciferase plasmid were co-transfected with 1 µg of nsp9 or nsp10 in HEK293 cells. At 48 h post-transfection, firefly luciferase activity was measured. (B) A combination of nsp9 and nsp10 failed to stimulate the mini-genome system. A combination of 0.2 µg of the mini-genome system plasmid DNA and 0.01 µg of the Renilla luciferase plasmid was co-transfected with 1 µg nsp9 and 1 µg nsp10 in HEK293 cells. At 48 h

post-transfection, firefly luciferase activity was measured.

Figure 4. Both ORF1a and ORF1b are essential for minus-strand RNA synthesis.

(A) ORF1a and ORF1b transfected alone failed to stimulate the mini-genome system.

A combination of 0.2 µg of the mini-genome system plasmid DNA and 0.01 µg of Renilla luciferase plasmid was co-transfected with 1 µg of ORF1a and 1 µg ORF1b in HEK293 cells. At 48 h post-transfection, firefly luciferase activity was measured. (B)

A combination of ORF1a and ORF1b stimulated the mini-genome system. A combination of 0.2 µg of the mini-genome system plasmid DNA and 0.01 µg of Renilla luciferase plasmid was co-transfected with 1 µg of ORF1a and 1 µg of ORF1b in HEK293 cells. At 48 h post-transfection, firefly luciferase activity was measured. *

$P < 0.05$ from three independent experiments.

Table 1. Sequences of the Primers Utilized in this Study.

Primers	Sequences
CMV-F	5'-GCAGATCTTTACATAACTTACGGTAAATGG-3'
CMV-R	5'-CGGAAAGATCGCCGTGTAATTGGAGATTCAGAGGAAG-3'
Luc-F	5'-CTTCCTCTGAATCTCCAATTACACGGCGATCTTTCCG -3'
Luc-R	5'-CAGGGCACAAAGTTCCAGCCACCATGGAAGA-3'
BGH-F	5'-TCTTCCATGGTGGCTGGAACCTTGTGCCCTG-3'
BGH-R	5'-GCCTCGAGGCTCTCCCCAGCATGCC-3'
ORF1a-F	5'-GCGGCCATGGAGGCCATGTCTGGGATACTTGATC-3'
ORF1a-R	5'-AAAGCGGCCCGCCTAGCAGTTTAAACACTGC-3'
ORF1b-F	5'-GCCTCGAGGTTTTAAACTGCTAGCCGCC-3'
ORF1b-R	5'-AAAGCGGCCGCTCAATTCAGGCCTAAAGTTGG-3'
nsp9-F	5'-GCGGCCATGGAGGCCCTAGCCGCCAGCGGCTTG-3'
nsp9-R	5'-AAAGCGGCCCGCCTACTCATGATTGGACCTG-3'
nsp10-F	5'-GCGGCCATGGAGGCCCGGAAGAAGTCCAGAATG-3'
nsp10-R	5'-AAAGCGGCCCGCCTATTCCAGGTCTGCGCAAATAGCGC-3'

A

HuN4 infectious clone

B

Mini-genome

C

Support plasmids

Fig.2, Tang and Fang et al., 2016

A

B

A

B

A

B