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Methods Compare for Rescued Porcine Circovirus Type 2 Viruses

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Abstract: Porcine Circovirus type 2 (PCV2), the essential causative agent of the Post weaning Multisystemic Wasting Syndrome (PMWS) has posed a grave threat to global swine industry in recent decades. Infectious molecular clone is the best way to get the purificated virus for pathogenesis study and inactivated vaccine produce. The purpose of this study was to compare and get the best way for PCV2 infectious molecular clone to rescue viruses. In this study, four PCV2 infectious molecular clone was constructed and transfected into PK-15 cells. IFA and Real-time PCR was used to detect the presence of PCV2. The IFA results revealed the precence of rescued viruses. The real-time PCR results showed that there were the most DNA copies with Ligation DNA transfection, the least DNA copies with pEASY-PCV2 transfection and almost the same with psk-2PCV2 and psk-PCV2 transfection. This assay afford the best way to construct and get PCV2 infectious molecular clone for further research.

Key words: Rescue, porcine circovirus type 2, infectious molecular clone, real-time PCR, cell

INTRODUCTION

Porcine Circovirus type 2 (PCV2), a small nonenveloped virus with a single-stranded circular DNA genome of approximately 1.7 kb in size in the genus *Circovirus*, family Circoviridae is the causative agent of Post weaning Multisystemic Wasting Syndrome (PMWS), clinical signs including lethargy associated with growth retardation, skin pallor, hyperthermia, enlargement of inguinal lymph nodes and immunosuppression to co-infect with other bacteria and virus (Tischer *et al*., 1982; Gillespie *et al*., 2009). PCV2 has caused a serious economic problem in the global swine industry in recent decades. To prevent PMWS, vaccines especially whole virus inactivated vaccine played an important role. Infectious molecular clone is the best way to get the purificated virus for pathogenesis study and inactivated vaccine produce. Accordingly, this study aimed to compare and get the best way for PCV2 infectious molecular clone to rescue viruses.

MATERIALS AND METHODS

Primer P1 5'-GAACCGCGGGCTGGCTGAACTTTTG AAAGT-3' and primer P2 5'-GCACCGCGGAAATTTCT GACAAACGTTACA-3' were used to amplify a 1.7 kb long region of the PCV whole genome as described by Fenaux *et al*. (2002). The PCV2 infectious clone was constructed as previously described by Li *et al*. (2009).

PK-15 cells, free of PCV1 contamination were seeded into six well tissue culture plates (1×10^5cells/well) and grown to approximately 70-90% confluency. After one wash with PBS, cells were transfected with psk-2PCV2 (two copys of PCV2 cloned into the pBluescript II SK (+) Vector System, Invitrogen, USA), psk-PCV2 (one copy of PCV2 cloned into the pBluescript II SK (+) Vector System), pEASY-PCV2 (one copy of PCV2 cloned into the pEASY-Blunt Simple Cloning kit, Transgen biotech, China) and ligation DNA (one copy of PCV2 cyclization) individually using Lipofectamine 2000 (Invitrogen, USA) according to the manufacturers' protocol. After 9 h post transfection, the liquid was discarded and the cells were maintained at 37°C with 5% CO_2 in Minimum Essential Medium (MEM) (Gibco) and 2% heat-inactivated FBS (Gibco). After 48 h post transfection, the Immunofluorescence Assay (IFA) and real-time PCR was used to detect the presence of PCV2. Plasmid pSK was used as negative control.

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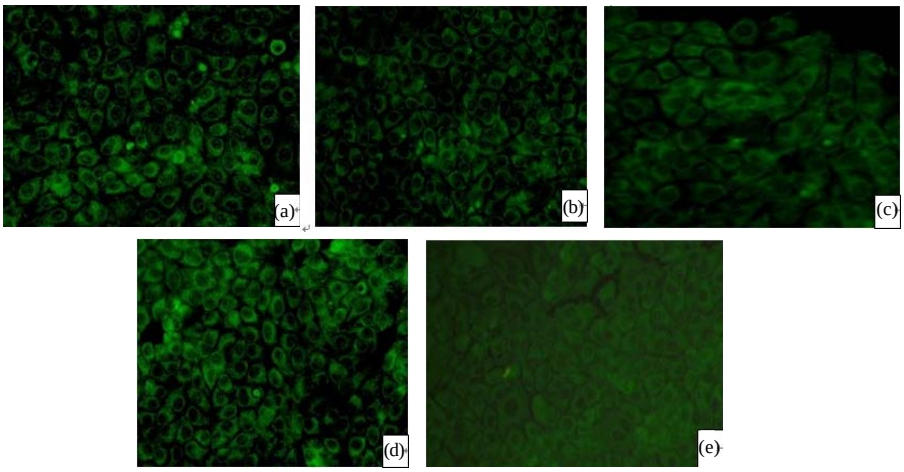

Fig. 1: The immunofluorescence assay result of rescued viruses (200x). a) psk-2pcv2; b) psk-pcv2; c) pEASY-pcv2; d) Ligation DNA and e) psk; The virus-transfected cells stained green and the ligation DNA groups had the most cells stained while control cells transfected plasmid pSK showed no staining

RESULTS AND DISCUSSION

Monoclonal antibody 3D4 against PCV2 Cap protein (1:100 dilution) and goat anti-mouse lgG (1:100 dilution, Invitrogen, USA) were used to do the IFA. After 48 h post transfection, the virus-transfected cells stained green and the ligation DNA groups had the most cells stained while control cells transfected plasmid pSK showed no staining. The results revealed the precence of rescued viruses (Fig. 1).

The virus-transfected cells DNA were extracted by the proteinase K method according to the instructions (Sambrook and Russell, 2001). Plasmid pEASY-PCV2 was serial 10-fold diluted as the positive standard and the real-time PCR was performed as previously described by Li *et al.* (2013). The DNA copies of rescued viruses were calculated by Roche 480 II Software. There were the most DNA copies with Ligation DNA transfection, the least DNA copies with pEASY-PCV2 transfection and almost the same with psk-2PCV2 and psk-PCV2 transfection (Fig. 2). The samples and the positive standard were all repeated three times.

The genome of PCV is circular, single-strandedDNA. There are two open reading frames, ORF1 and ORF2 which diverge from the Ori. ORF1 is a clockwise virion-strand rep-gene region that encodes two replication proteins (Rep and rep') and ORF2 is a complementary-strand cap-gene region that encodes the capsid protein (Cap). During DNA synthesis, the PCV origin of DNA replication (Ori) which contains a pair of inverted repeats (palindrome), exists in a destabilized four-stranded configuration (the melting-pot model) and permits both the palindromic-strand and the

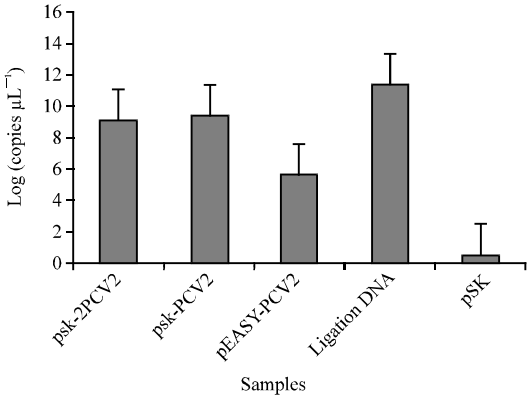

Fig. 2: DNA copies of rescued viruses after transfection. Ligation DNA has the most DNA copies, pEASY-PCV2 has the least DNA copies; plasmid pSK was used as negative control

complementary-strand to serve as templates simultaneously for initiation and termination, rep and rep' are essential for virus DNA replicationvia the Rolling-Circle Replication (RCR) mechanism (Cheung, 2012). The pBluescript II SK (+) are cloning vectors designed to simplify commonly used cloning and sequencing procedures including the construction of nested deletions for DNA sequencing, generation of RNA transcripts *in vitro* and site-specific mutagenesis and gene mapping. The pEASY-Blunt plasmid are blunt terminus cloning vectors. When inserted to plasmid to construct infectious clone, the PCR products of PCV2 whole genome were disconnection but it was end to end in the ligation DNA methods, the most appropinquity to virus infection. This method gotten the most DNA copies and it has been used in PCV2 research (Guo *et al.*, 2011).

CONCLUSION

The four methods were compared and the ligation DNA method was the best to rescue PCV2 viruses.

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