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LIGATION OF E2 GENE WITH REPLICASE BASED EUKARYOTIC pSinCMV VECTOR AND ITS TRANSFORMATION STUDY

Nitin Sharma*,Purshotam Kaushik[1], Anant Rai[2]

*Corresponding author: email- nitinsharma.com@gmail.com, Mb no,-09897857585

[1]Dept of Microbiology, Gurukul Kangri Vishwavidyalaya-Haridwar

[2]Institute of Biotechnology & IT, 197, Mudia Ahamadnagar, Pilibhit Road, Bareilly (UP).

ABSTRACT: The E_2 gene of classical swine fever virus (CSFV) is already cloned in pVAX1 vector. In the present study we have released the E_2 gene insert from the pVAX1.CSFV.E_2 using plasmid isolation, RE digation and legated with pSinCMV vector. Prepared the competent cell to transformation of Ligated product in E.*coli* DH5α. Make the L.B. Agar plate and the large number of colonies (approximately 25) were picked from the overnight grown transformants. The individual colonies were inoculated in fresh ampicillin (50 µg/ml) containing LB broth and allowed to grow for large scale production for further experiments. This can be used for expression and immunological studies.

Key words: Classical Swine Fever Virus, Transformation, E_2 gene, Restriction enzyme.

INTRODUCTION

Classical swine fever (CSF), also known as hog cholera, is a highly contagious, multisystemic and hemorrhagic viral disease, included in the list of diseases notifiable to the OIE (www.oie.int), distributed almost world-wide and is considered the most economically important but vaccinepreventable disease of swine in areas of intensive pig farming. Natural host of classical swine fever virus are members of the family Suidae, which include domestic pigs and wild boars (Depner et al., 1995; Laddomada, 2000). A considerable problem is the survival CSF virus in wild pig population, which is considered to be the potential source of the infection.

Genomic RNA possess single ORF. Initially a polyprotein is formed which is further cleaved cotranslationally and post-tanslationally by the host and viral genome encoded proteases to yield four structural (C protein, Erns, E1 and E2) and seven non structural proteins. Erns, E1 and E2 are the viral envelope glycoprotein. After infection with CSFV, antibodies are raised against the structural glycoproteins E2 and Erns and non structural protein NS3 (Paton et al., 1991; Terpstra, 1988). Antibodies against Erns and NS3 have only low neutralizing capacity or none at all (Rumenapf et al., 1991; Konig et al., 1995).

E2 glycoprotein contains most of the known humoral and cell mediated protective determinants of CSFV (Ceppi et al., 2005), highly immunogenic against which most of the neutralizing antibodies are induced and the only one capable of conferring protection against CSFV challenge (Rumenapf et al., 1991; Hulst et al., 1993).

MATERIALS AND METHODS

Vector: The replicase based pSinCMV vector (Nagarajan, 2005), was used.

CSFV E_2 gene

The E_2 gene already cloned in pVAX1 vector was used as the candidate gene, the sequence accession no. EU857642.

Host Bacterial strains

Escherichia coli (E.coli) DH5α (Proteges,Madison,USA) host strain was used for transformation experiments.

Preparation for E_2 gene

pVAX1.CSFV.E_2 recombinant plasmid containing E_2 gene insert was revived in LB broth and plasmid DNA was isolated (Sambrook and Russell 2001). Restriction endonuclease digestion of recombinant plasmid pVAX1.csfv.E_2 with PmeI enzyme was done to release the E_2 gene insert. This enzyme create blunt end. The size of E_2 gene ORF is 1.125 Kb.

pSinCMV vector preparation and ligation

StuI was use to create blunt end using 50 µl reaction mix. A 10 µl reaction mixture was for blunt end ligation containing T4 DNA ligase (Fermentas) 1 µl, pSinCMV Vector 2 µl, CSFV E_2 gene 5 µl, ligation buffer (10X) 1µl, nuclease free water 1 µl. The reaction mixture was incubated overnight at 14°C. The linearised plasmid was checked and quantitated on 1% agarose gel electrophoresis. The size of pSinCMV vector is 10.779 Kb.

Preparation of competent cells and Tansformation

Took the 500 µl and equal volume of ice-cold 2xTSS is added in tube and the cell suspension mixed gently. Then 2 µl Ligated DNA and mixed the 100 µl cell suspension (competent cell), mixture incubate for 5-6 min at 4°C, add the 0.9 ml of SOC, incubate 37°C with shaking for 1 hr to allow expression of the antibiotic-resistance gene. Transformants are selected by standard methods (Chung et al., 1989).

Screening of recombinant clones

A large number of colonies (approximately 25) were picked from the overnight grown transformants. The individual colonies were inoculated in fresh ampicillin (50 µg/ml) containing LB broth and allowed to grow for 18 to 24 hours. This culture can be use for immunological studies.

RESULTS

Subcloning of csfv E_2 gene in pSinCMV.csfv. E_2

The csfv E_2 gene cloned in pVAX1 vector was released by digesting with PmeI enzyme then makes the 2 fragments one is vector's size 2979 bp and second is 1119 bp of E_2 gene (**Fig. 1**). This PmeI enzyme create blunt end. The pSinCMV vector was linearized by digesting with StuI enzyme for used to create blunt end size 10779 bp **(Fig. 2),** gel eluted, purified by phenol chloroform precipitation method. Blunt end ligation was done and ligated vector was transformed in *E.Coli* (DH5α) cells. Transformed product was spread in L.B. agar plate, after incubated over night at 37°C blue-white colony seen **(Fig. 3).**

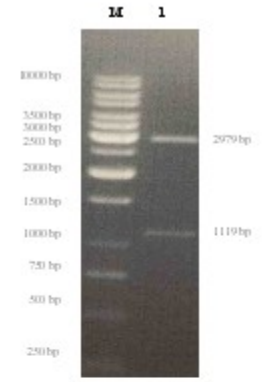

Fig 1. Restriction digestion of pVAX1.scfvE2 plasmid with PmeI enzyme
Lane M- Marker 1 Kb Ladder
Lane 1 Digestion with PmeI releasing a product of 2979 bp & 1119 bp

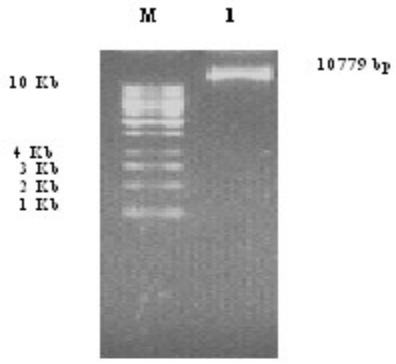

Fig 2. Preparation of pSinCMV vector
Line M - 1 Kb DNA Marker
Line 1 - pSinCMV vector digestion with StuI enzyme

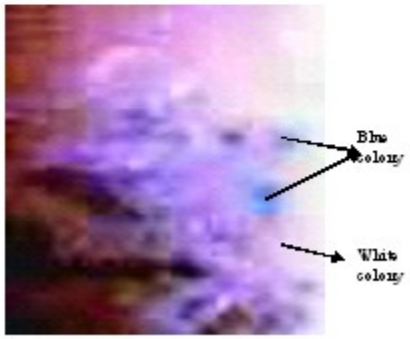

Fig 3. Transformation showBlue & White colony in plate

DISCUSSION

E_2 gene has become the main candidate for the development of DNA vaccine against CSFV. We have tried to transform the E_2 gene in replicase based eukaryotic vector namely pSinCMV.

pSinCMV is an alpha virus (Sindbis virus) based plasmid vector which serves as negative strand once it enters into the host cell. There after entering into the nucleus it is transcribed by host RNA polymerase enzymes from CMV promoter into a full length RNA transcript. This full length transcript then acts as positive sense alpha virus which in turn is translated in cytoplasm to form replicase protein. This protein serves as RNA dependent RNA polymerase enzyme and forms negative sense RNA from positive sense transcript. From this negative sense strand full length as well as smaller fragments from subgenomic promoters are transcribed which in-turn is translated into proteins.

Since the cloned insert is downstream to the subgenomic promoter, the translated proteins represents our target proteins. The subgenomic promoter of alpha virus is very strong so that it makes large number of target mRNA from the sequence downstream to it. Replicase based vectors are superior over other conventional vectors in terms of its lower requirement of dose of immunization (Xiong et al., 1989; Hariharan et al.,1998, Berglund et al., 1998, Leitner et al., 2000).

In the transformation blue-white colony showed the E_2 gene is transformed in pSinCMV vector on the plate. Further we can use of pSinCMV.csfv.E_2 for large scale production in L.B. broth and after confirmation by PCR and sequencing can be use in expression and immunological study.

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