Construction of Prokaryotic Expression Vectors Bearing S Gene of Isolate TH-98 from Transmissible Gastroenteritis Virus

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Abstract: Sa fragment containing major antigenic sites A, B, C and D of S gene from transmissible gastroenteritis virus (TGEV) Chinese isolate TH-98 was purified with *Eco*RI and *Kpn*I from recombinant pUC-S and cloned into prokaryotic expression vector pProExHTb. The recombinant SaPRO was identified with restriction enzyme (RE). Sa derived from recombinant SaPCI was inserted into *Eco*RI and *Not*I sites of expression vector pET30c with the similar DNA recombination technique. The construct designated SaPET was transformed into *Escherichia coli* (*E. coli*) BL21. Then, SaPET was digested with *Xho*I and ligated in order to gain the recombinant SasPET carrying B, C and D sites of S gene, which was also transformed into BL21. The acquisition was subcloned into corresponding sites of another prokaryotic expression vector pGEX-6P-1 after digestion with *Eco*RI and *Xho*I. The verified recombinant Sas6P-1 was re-transformed into BL21. [Life Science Journal. 2006;3(1):63 - 66] (ISSN: 1097 - 8135).

Keywords: TGEV; S gene; prokaryotic expression vector; construction

1 Introduction

Transmissible gastroenteritis virus (TGEV) is one of the important pathogens for virus diarrhea of swine (Yin, 2005). Spike(S) protein is the major immunogen among four major structural proteins (S, M, N and sM) of TGEV (Krempl, 1997). Particularly the 5′ end half of S gene encoding the major antigenic sites are critical for inducing neutralizing antibodies (Gebauer, 1991; Tuboly, 1994). To gain the S protein is a prerequisite for the diagnosis, prevention and treatment of transmissible gastroenteritis (TGE). The prokaryotic expression system shows some significant advantages over other systems in production cost, technology and cycle etc. However, the limited expression capacity for foreign genes and the different expression efficiency of different vectors used in this system are the disadvantages.

For these reasons, we constructed several recombinant plasmids encoding the 5′ end half of S gene or major antigenic site fragment using different prokaryotic expression vectors. These plasmids have been transformed into *Escherichia coli* (*E. coli*). This study provided the important materials for S protein expression and comparison of vector expression efficiency.

2 Materials and Methods

2.1 Vector, host cells, tool enzymes and primers

Recombinant plasmids pUS-C and SaPCI respectively containing full-length and 5′ end half of S gene of TGEV have been constructed in our laboratory (Ren, 2003). Prokaryotic expression vectors, pProExHTb (GIBCO), pET30c (Novagen) and pGEX-6P-1 (Amersham Biosciences) were commercially purchased. TG1, DH5α and BL21 competent cells were also commercially obtained. Tool enzymes were purchased from Takara Biotechnology Company (Dalian, China). Primers, PS3: 5′-TACAGTGAGTGACTCGAGCT-3′ (1242) and PR3:5′-GGTGTTGTTGTCCAATGTG-3′ (2408) were used for nested PCR (The figures in brackets are the corresponding or complementary position of S gene).

2.2 Construction of expression plasmids containing S gene of TGEV

Recombinant pUC-S was digested with *Eco*RI and *Kpn*I to obtain the 5′ end half of S gene named Sa. Sa was ligated with vector pProExHTb digested with the same enzymes and transformed into DH5α cells. A positive recombinant named SaPRO

was selected in LB agar plate containing appropriate antibiotic and identified with restriction enzyme (RE). Recombinant SaPCI was digested with *Eco*RI and *Not*I, and a linearized fragment encoding the Sa region was cloned into pET30c vector using the similar method described above. A positive recombinant was named SaPET after the identification with RE and by nested PCR. To facilitate further inducible expression, SaPET was transformed into BL21 cells. A strain of plasmid-containing cells was selected.

DNASIS software analysis indicated there were two *Xho*I sites located in near nucleotide 1120 of S gene and in the multiple cloning sites after Sa fragment in SaPET. Therefore we digested SaPET with *Xho*I and a resulting fragment containing vector and 5′ end S gene about 1100 base pair (bp) in length was ligated and transformed into DH5α cells. A recombinant named SasPET was identified with RE and re-transformed into BL21 cells. SasPET was digested with *Eco*RI and *Xho*I, and the foreign fragment was cloned into vector pGEX-6P-1. A positive recombinant identified with RE was named Sas6P-1. All the recombinants were sequenced to confirm the authenticity of the sequence.

3 Results

3.1 Identification of SaPRO with RE

SaPRO was digested with RE according to the physical map of the vector pProExHTb and Sa sequence. The digested fragments were visualized by 0.8% agarose gel electrophoresis. This result was identical to theoretical calculation (Figure 1).

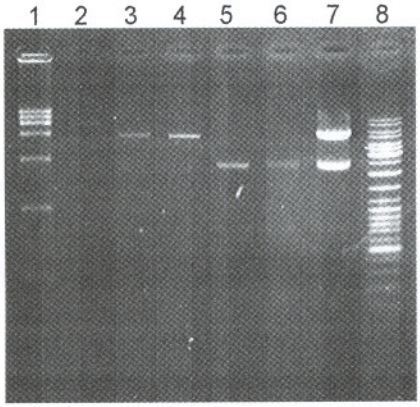

Figure 1. Identification of SaPRO with RE
Lane 1: DL 15, 000 DNA marker (TaKaRa, China).
Lane 2: SaPRO digested with *Xho*I, of about 7.1 kb.
Lanes 3 and 4: Vector pProExHTb linearized with *Eco*RI and *Kpn*I, of about 4.8 kb.
Lanes 5 and 6: Fragment Sa digested with *Eco*RI and *Kpn*I, of about 2.3 kb.
Lane 7: SaPRO digested with *EcoR* I and *Kpn*I, of about 2.3 kb and 4.8 kb respectively.
Lane 8: DNA Ladder (MBI Fermentas).

3.2 Identification of SaPET

Recombinant SaPET was identified with RE and primer-specific PCR. The agarose gel electrophoresis showed that Sa has been inserted pET30c vector (Figure 2).

Figure 2. Identification of recombinant SaPET
Lane 1: Identification of SaPET by nested PCR, of about 1.2 kb.
Lane 2: DL 15,000 DNA marker.
Lane 3: SaPET digested with *Eco*RI, of about 7.7 kb.
Lane 4: SaPET digested with *Xho*I, of about 7.7 kb.

3.3 Identification of SasPET

SasPET was analyzed with RE, and the result indicated that 5′ end fragment of S gene about 1100 bp in length has been cloned into pET30c (Figure 3).

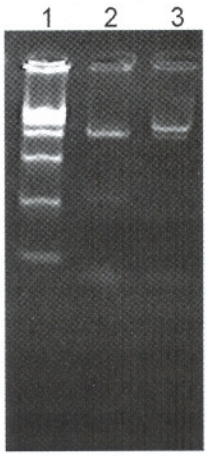

Figure 3. Identification of recombinant SasPET
Lane 1: DL 15,000 DNA marker.
Lane 2: SasPET digested with *Eco*RI and *Xho*I, of 1.1 kb and 5.4 kb respectively.
Lane 3: SasPET digested with *Eco*RI, of 6.6 kb.

3.4 Identification of Sas6P-1

Sas6P-1 was digested with RE, and the analysis result of agarose gel indicated the correct insertion of fragment of interest (Figure 4).

3.5 Sequencing analysis

The sequencing report verified there was no nucleotide insertion and deletion in the S gene used in this study and it was identical to the published sequence (GenBank™ accession number AF494337).

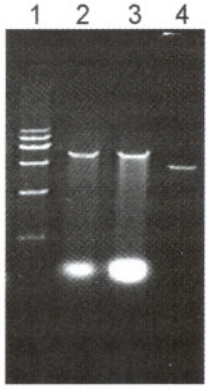

Figure 4. Identification of recombinant Sas6P-1
Lane 1: DL 15,000 DNA marker.
Lane 2: Sas6P-1 digested with *Bam*HI, of about 6.1 kb.
Lane 3: Sas6P-1 digested with c*Eco*RI, of about 6.1 kb.
Lane 4: Vector pEGX-6P-1 linearized with *Eco*RI and *Xho*I, of about 4.9 kb.

4 Discussion

Gene expression system(GES) is very useful in genetic engineering field. Currently, prokaryotic, yeast and eukaryotic expression systems are frequently used GES. In which reformed *E. coli* is very popular for protein production in large scale due to their rapid growth rate, facilitation for continuous fermentation and relatively low cost. However, the genome of *E. coli* is well characterized and a lot of cloning vectors are available (Grisshammer, 1995).

The initial purpose of this study is to express 5′ end half of S gene using *E. coli*. However the expression level of foreign gene in *E. coli* might be influenced by the type of prokaryotic expression vector and length of gene of interest (Francisco, 1993; Wels, 1995; Jahn, 1995).

Therefore, we took full advantage of different vectors and different fragments of S gene to construct various recombinant plasmids. At the same time, we tried to decrease the length of S gene under the circumstance of retaining the major antigen sites in order to increase the probability of successful expression of S gene. The usage of different vectors enables us to make multiple choices for future protein purification.

In the process of construction, the sequenced recombinants were utilized to give gene of interest, which enhanced the positive recombination ratio and decreased possible mutation might derived from PCR amplification. However, it should be noted that open reading frame (ORF) of S gene must be correct after inserting into suitable vectors to prevent the shift of ORF. Most vector we used here are high efficient vectors that can express gene of interest in the form of fusion protein, decrease the toxicity to host cells and facilitate the downstream purification.

To sum up, we constructed four recombinant plasmids encoding the major antigenic sites of TGEV. These positive *E. coli* strains might be induced to express the gene of interest, which provided the useful materials for further related research.

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