

Designing a recombinant Bacmid construct of HCV core+1 in Baculovirus expression system

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ABSTRACT

Background and Objectives: Hepatitis C virus (HCV) chronically infects around 200 million people worldwide and frequently causes liver cirrhosis and hepatocellular carcinoma. Rapid detection of this virus results in decreasing the distance between infection and initiation the anti-viral treatment, and may prevent most of the undesirable consequences. The new detected HCV protein "Core+1" made from the ribosomal frame shift in Core region is an important candidate for diagnostic tools. This study was conducted to design a recombinant Bacmid plasmid expressing the HCV 1a Core+1 sequence in the Baculovirus expression system for further diagnostic applications.

Materials and Methods: The HCV Core +1 gene was amplified by PCR using the pcDNA-HAF recombinant vector that contained the Core+1 sequence from HCV genotype 1a as a template, and the specific primers with 2 restriction sites for *Nco* I and *Xba* I restriction enzymes. The PCR product was cloned in *Xba*I/*Nco*I restriction sites of the linearized *pFastBac-HTB* vector and evaluated by using those restriction enzymes and sequencing. Then the recombinant *pFastBac-HTB* vector was transformed in DH10Bac and the result was screened and confirmed by X-Gal discrimination and PCR.

Results: The HCV 1a Core+1 was successfully amplified and the PCR product was confirmed by using the related restriction enzymes and sequencing. Cloning of *pFastBac* vector with the purified PCR product of HCV Core+1 was confirmed. Finally, the recombinant Bacmid was successfully transformed in DH10Bac.

Conclusion: The recombinant Bac-Core+1 expression vector is considered as an important tool to transfect the sf9 cell line and expression the Core+1 protein.

Keywords: Hepatitis C Virus, HCV Core+1, *pFastBac* vector, Bacmid expression vector, Baculovirus expression system.

INTRODUCTION

Hepatitis C virus belongs to the genus Hepacivirus in Flaviviridae family that contains a 9.6 kb single

stranded positive RNA genome with a single open reading frame (1, 2). This virus is a major cause of chronic liver diseases, including steatosis, cirrhosis and hepatocellular carcinoma (1, 3). It has been proposed that HCV infection is also associated with insulin resistance and type 2 diabetes mellitus (1, 3). This small enveloped virus encoding a polyprotein as a precursor which is processed by host and viral proteases into at least 10 different proteins (1, 3). There is a second functional Open Reading Frame (ORF), within the core gene, encoding an additional protein named alternative reading frame protein

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(ARFP), frameshift (F) or Core+1 (to indicate its position) (3, 4). Several studies have shown that HCV F protein has been produced from reading frame by more than one type of coding event (4, 5). The effect of this viral protein on virus replication remains elusive. However there are some evidences about immune response against this protein through measuring the anti- Core+1 antibody in HCV infected patients such as hepatocellular carcinoma (6, 7). Core+1 is produced through the ribosomal frame shift in core region so, these two viral proteins have the overlapped ORFs (5, 8). Since HCV core protein is a multifunctional protein, Core+1 may contribute to some functions attributed to the core protein (9). Several studies reported the presence of anti-F protein antibodies in some HCV-positive patients (7, 10, 11). However, the exact role of this virus product in induction of immune response and virus – associated pathogenesis remains controversial (6, 10, 12). Consideration of different expression systems, to find the most efficient and safest with lower costs and labor intensive, is an important context in modern biotechnology. The most described organism in this content is *E.coli* expression system with quick growth in simple media, distinct biological process and high yield of recombinant protein expression (13, 14). At this point, some enzyme-linked immunoabsorbent assays has been developed using *E.coli* expressed purified recombinant HCV Core+1 protein (10, 15). Lack of post -transcriptional modifications in eukaryotics proteins results in designing other expression systems such as Baculovirus expression system (8, 16). The Baculovirus system has become one of the most powerful eukaryotic systems for recombinant protein expression since 1983. This expression system provides the post translational processing, and folding of recombinant proteins that are highly similar to those occurred in natural infection in a eukaryotic host (16-18). This study was conducted to design a recombinant Bacmid plasmid expressing the HCV 1a Core+1 sequence in Baculovirus expression system for further diagnostic applications.

MATERIALS AND METHODS

PCR. The HCV Core +1 gene was amplified by PCR in 50 μ l reaction mixture using the pCDEF-HAF recombinant vector (kindly gifted from Professor Jing-hsiung Ou) containing the Core+1 sequence

from HCV genotype 1a as a template. 1 μ l of this template DNA was added to the PCR mixture which contained 1 μ of 20 pmol of each forward 5'-ATCCAT-GGGCACGAATCCTAAACCTC-3' and reverse 5'-CATCTAGATTATCACGCCGTCTTC-CAGAAC-3' primers, (the underlined nucleotides demonstrate *Nco* I and *Xba* I digestion sites).

Five μ l of 10X PCR reaction buffer, 1 μ l dNTP mix and 0.5 μ l of PFU DNA polymerase (Fermentas Life Science, Lithuania), and 40.5 μ l dDW. This reaction was amplified in a Master cycler Gradient (Eppendorf, Germany) using the following conditions: initial denaturation at 94°C for 5 minutes, followed by 35 cycles at 94°C for 30 second, 55°C for 30 second and 72°C for 35 second and a final extension phase at 72°C for 5 minutes. The PCR products were run on agarose gel electrophoresis for further analysis and subsequently purified and sequenced using ABI Prism automated sequencer Pairwise alignment was done by comparing the sequence of PCR product with Core+1 sequence of the pCDEF-HAF recombinant vector.

Cloning of the Core+1 genes. The Core+1 segment was gel-extracted using the PCR product purification kit (QIAquick Gel Extraction Kit, QIAGEN GmbH) and was cloned through the *Xba*I and *Nco*I sites into the pFastBacHTB donor vector (Fermentas Life Science, Lithuania), making the recombinant pFastBac-Core+1 plasmid. The fidelity of cloning was evaluated using the relevant restriction enzymes and sequencing of PCR product as described in previous section.

Construction of recombinant Bacmid. *E. coli* strain DH10Bac was transformed using the pBac-Core+1 recombinant donor plasmid. To perform site-specific transposition of the Core+1 fragment from the donor plasmid to the Bacmid DNA which already available in DH10Bac *E. coli* cells, the transformed DH10Bac cells were transferred to the Luria-Bertani broth (LB) agar plate containing Gentamicin (7 μ g/ml), Kanamycin (50 μ g/ml), Tetracycline (10 μ g/ml), X-gal (100 μ g/ml) and isopropylthio- β -galactoside (IPTG, 40 μ g/ml) and incubated at 37°C for 48 h.

Afterward, the largest white colonies were restreaked on the new LB agar plates containing the mentioned reagents to reconfirm their white phenotype. Finally, the confirmed white colonies were

overnight cultivated in LB broth medium containing the mentioned antibiotics and subjected to Bacmid extraction using plasmid extraction mini kit according to the manufacturer's instructions (AccuPrep® Plasmid Extraction Kit, Bioneer Cooperation, Korea). In order to confirm the extracted recombinant Bacmid, PCR was performed using universal M13 forward and reverse primers and the recombinant Bacmid contains pFastBac-HCV Core+1, the recombinant Bacmid - pFastBac and the non-recombinant Bacmid without pFastBac (as separate templates respectively) as instructed by the manufacturer (Fermentas Life Science, Lithuania).

RESULTS

Core+1 amplification, cloning and confirmation. HCV Core+1 sequence from genotype 1a was amplified successfully through PCR by using specific primers and the amplified fragment (483bp) was cloned into pFastBacHTB plasmid. The fidelity of the cloning was confirmed by restriction digestion and sequencing (Fig. 1).

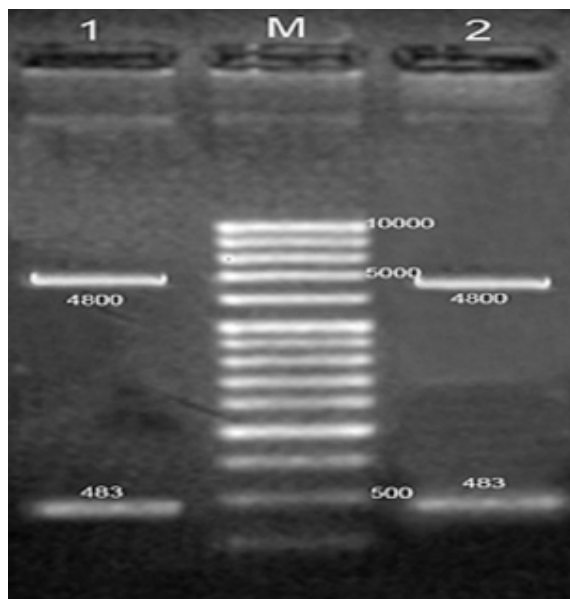


Fig. 1. Digestion of HCV (1a) Core+1 PCR product. Lane M: 1Kb DNA Ladder, Lane (1,2): pFastBacHTB donor plasmid (4800bp), Core+1 fragment: (483bp).

Construction of the recombinant Bacmid DNA. *E-coli* DH10Bac strain was transformed using the recombinant pFastBac- HCVCore+1 vector and Blue/white screening was performed. The extracted recombinant Bacmid was verified by PCR using the M13 universal primers and a 2856 bp amplified product was observed (Fig. 2). There was a recombinant Bacmid -pFastBac (Lane 3,4) as the negative control (2373), and also, a 273 bp fragment (Lane C) represented a PCR control for the non-recombinant Bacmid alone (without pFastBac) (Fig. 2).

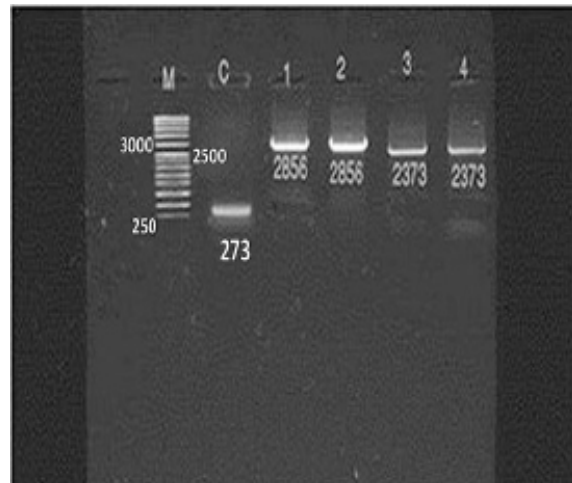


Fig. 2. Confirmation of the recombinant Bacmid. Lane M: 1Kb DNA Ladder, Lane C: Non-recombinant Bacmid alone without pFastBac (273bp); Lane (1,2): recombinant Bacmid contains pFastBac-HCV Core+1 (2856bp); Lane (3,4): recombinant Bacmid - pFastBac (2373bp).

DISCUSSION

HCV is classified into seven major genotypes, with more than 80 subtypes that amongst them subtypes 1a, 1b, 2a, 2b, 2c, and 3a represent a worldwide epidemic including Iran (1, 3). HCV has a single ORF and its ssRNA genome encodes three structural (Core, E1, and E2) and seven non-structural (P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) proteins (1, 2). An additional HCV protein, the F protein results from a -2/+1 frameshift at the HCV AUG initiator codon of the polyprotein so, this overlapping ORF lacks an AUG codon near its 5' end (4, 19). Core+1 with 162 codon is a highly conserved protein that expressed during the natural infection, and its expression from the core

coding sequence is independent of the downstream E1 region so, it is proposed that it is a truncated core protein (9). Shorter forms of it ranging from 126 to 155 codons in length and are also conserved. However it has been revealed that the length of the F protein is genotype specific (4, 9). According to these findings expression an intact form of F protein is very important especially when it used to develop the laboratory diagnostic methods such as ELISA. There are some reports of expressing the Core+1 protein in different expression systems other than Baculovirus expression system such as *E. coli* and mammalian expression systems (9, 20). Also, in some of these investigations the expressed Core+1 proteins were used in immunoprecipitation /ELISA tests to study its antigenic properties (9). Recombinant Baculovirus expression systems are one of the important tools for highly efficient gene expression through infection of insect cells (16, 17). This system is used for expression of many mammalian recombinant proteins with high efficiency. It has some significant advantages like the modifications, processing and other features of natural mammalian host cells with high yield of products.

This study was conducted to design and make a recombinant Baculovirus vector (Bacmid) contains the HCV Core+1 sequence derived from the genotype 1a for further investigations. There are some studies that HCV structural genes were expressed in Baculovirus expression system (21).

In conclusion, the HCV Core+1 candidate to Bacto-Bac system and successfully cloned. It was transferred to Bacmid by transposition mechanism. Finally, we suggest the use of this Bacmid to express the Core+1 protein for further studies.

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