Original Article

# Cloning and Expression of Hepatitis C Virus Core Protein in pGemex-1 Expression Vector

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**Background:** Hepatitis C virus is one of the main causes of chronic hepatitis in developing countries. There are 170 million affected people around the world as reported by the World Health Organization. The treatment of hepatitis C is not successful in most cases; it is extremely costly, and requires prolonged therapy, therefore it is desirable to develop a vaccine to prevent the spread of hepatitis C virus.

**Methods:** Hepatitis C virus RNA was extracted from a hepatitis C virus-infected serum sample. cDNA was synthesized and the hepatitis C virus core gene was amplified by polymerase chain reaction. The polymerase chain reaction product was cloned in pGEMEX-1 expression vector and expressed in *Escherichia coli* BL21 strain with DE3 (a λ prophage carrying the T7 RNA polymerase gene) host by induction of promoter using one millimolar isopropyl β-D-thiogalactopyranoside in laboratory scale. Induced lysate cells were electrophoresed on SDS-polyacrylamide gel.

**Results:** A protein band was detected in induced cells in comparison with non-induced cells. Expressed protein was confirmed by gel diffusion and dot blot analysis using induced lysate cells as antigen and hepatitis C virus-infected serum as antibody.

**Conclusion:** In present study, we have provided a recombinant plasmid based on hepatitis C virus core gene.

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**Keywords:** Gene expression • hepatitis C virus



## Introduction

Hepatitis C virus (HCV) is one of the main causes of chronic hepatitis in developing countries. It is estimated that 170 million people are affected around the world, as reported by the World Health Organization (WHO).[1] These persistently infected individuals are the source for most new infections. The treatment of hepatitis C is not successful in most cases, and is also extremely costly and long; therefore, it is desirable to develop a vaccine to prevent HCV infection.[2] The core gene of HCV is one of the most conserved regions of the HCV genome. This conservation extends across different genotypes, making it an ideal candidate for inclusion in a broadly protective DNA- based vaccine. The extreme conservation of these epitopes makes them less likely to be susceptible to escape mutations.[2]

The core protein of HCV appears to be a multifunctional protein that is involved in many viral and cellular processes.[3] van Pelt et al.[4] reported the effect of HCV core protein on DNA repair after ultraviolet (UV)-induced DNA damage. Cristofari et al.[5] reported that core protein chaperoned the annealing of complementary DNA and RNA sequences and the formation of the most stable duplex by strand exchange. Their results show that the HCV core is a nucleic acid chaperone that acts as retroviral nucleocapsid

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proteins and is involved at several stages of virus replication. Siavoshian et al.[6] reported results showing that the effects of the core, NS3, NS5A, and NS5B on cell proliferation were independent from p53 expression and that only the core protein induces the expression of both c-myc and p53. Shimoike et al.[7] suggested that the HCV core protein interacts with viral genomic RNA at a specific region to form nucleocapsids and regulates the expression of the HCV genome by interacting with the 5' untranslated region (UTR). The aim of this study was to clone the HCV core protein gene for use in recombinant HCV vaccine development.

## Materials and Methods

### Sample and RNA extraction

HCV RNA was extracted from an infected patient whose disease was confirmed by polymerase chain reaction (PCR) in the HCV genotyping project.[8] Viral RNA was extracted from 50 µL of serum with 200 µL RNX-plus buffer (Fermentas, Lithuania) according to the manufacturer's instructions. The mixture was incubated for five minutes at room temperature, and then 50 µL of chloroform was added to it before being centrifuged at 12000 rpm for 15 minutes at 4°C. Total RNA was precipitated by ethanol, and then dissolved in 10 µL of diethyl pyrocarbonate-treated water.

### cDNA synthesis

Reverse transcription (RT) was performed by incubating the template RNA (equivalent to 50 µL of serum) in a 20-µL reaction mixture containing 40 picomole (pmol) of specific antisense primer (Hcor R 5`- GGA TCC GGC TGA CGC GGG CAC AGT C- 3`), 100 units of RT enzyme (Fermentas Lithuania), 20 units RNasine (Fermentas, Lithuania), 1× RT buffer, and 0.2 mM deoxynucleotide triphosphates (dNTPs) for one hour at 42°C.[9]

### PCR reaction

PCR was carried out to amplify the HCV core gene fragment. The PCR reaction mixture contained 0.1 µg of synthesized cDNA, 0.1 mM dNTPs, 1.5 mM $MgCl_2$, 20 pmol of each of the forward and reverse primers (Hcor F 5`- GAG CTC ATA TG A GCA CGA ATC CTA AAC -3` and Hcor R 5`- GGA TCC GGC TGA CGC GGG CAC AGT C- 3`), and 1.25 units of Taq DNA polymerase in 50 µL of final volume. PCR reaction was carried out within 30 cycles; denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and elongation at 72°C for 40 seconds.[10] The PCR product was submitted to electrophoresis using 1.5% agarose gel, stained by ethidium bromide, and visualized under a UV transilluminator.

### Cloning

The HCV core PCR product was electrophoresed on 1.5% low melting point (LMP) agarose gel and the DNA band was sliced under long wave UV. DNA was extracted using the DNA purification kit (Fermentas, Lithuania). The extracted PCR product and EcoRV blunt digested pBR322 were 3` tailed using dATP and dTTP respectively by dNTP.[11,12] The 3`T-tailed PCR product was ligated into plasmid via the T/A cloning method,[13] and transformed into *E. coli* XLI-blue strain[14] which contained recombinant plasmid that was screened by insertion inactivation of a tetracycline-resistant gene and named pBKC1. The recombinant plasmid was digested by SacI and BamHI restriction enzymes established on the 5` end of the forward and reverse primers respectively, and electrophoresed on 1.5% LMP agarose gel. The inserted DNA was sliced and recovered using the DNA purification kit.

Recovered insert DNA was subcloned in pGemX-1 expression vector in SacI and BamHI recognition sites. The reaction was transformed in *E. coli* XL1 blue competent cells and the positive colonies containing recombinant plasmids were mass cultured in Luria Bertani (LB) medium. The recombinant plasmid, extracted[15] and confirmed by restriction analysis, was designated pBKC84.

### Gene expression

Expression was performed as described previously[16] with some modifications. Briefly, the *E. coli* BL21 strain with DE3 (a λ prophage carrying the T7 RNA polymerase gene) was transformed with pBKC84 (containing the core gene) and selected on LB agar containing 50 µg/mL of ampicillin. The transformant was inoculated into 3 mL culture tube containing modified yeast tryptone (YT) medium (1.2% bacto trypton, 2.4% yeast extract, 0.04% glycerol, and 1% M9 salts: 6.4% Na2HPO4 - 7H2O, 1.5% KH2PO4, 0.025% NaCl, 0.05% NH4Cl) and was allowed to grow overnight at 37°C in a shaker incubator at 160 rpm. The following day, the cultured bacteria was inoculated into a 50 mL flask and incubated at 37°C in a shaker at 200 rpm.

Cultures in logarithmic phase (at OD600 of 0.6) were induced for six hours with 1 mM isopropyl β-D-thiogalactopyranoside (IPTG). After induction, cells were lysed in 5x sample buffer [100mmol Tris HCl pH 8, 20% glycerol, 4% sodium dodecyl sulfate (SDS), 2% beta mercapto- ethanol, 0.2% bromo phenol blue] and analyzed with 12% SDS-polyacrylamide gel.[17] The gel was stained with Coomassie brilliant blue R-250.[18] Uninduced control culture was analyzed in parallel.

### Serologic assay

Gel diffusion was done using induced lysate cells as antigen and HCV-infected serum as antibody loaded on 1% agarose gel in phosphate buffered saline (PBS) and incubated over night at room temperature.[19]

### Dot blot analysis

Lysate-induced cells were blotted on nitrocellulose membrane. Nitrocellulose membrane blot was reacted by primary antibody (HCV-infected serum) and then by secondary antibody (human anti-IgG peroxidase conjugated), and subsequently detected by its substrate.[20]

## Results

### PCR amplification

Synthesized cDNA was amplified by PCR using designed HCV core specific forward and reversed primers. The PCR product was electrophoresed on 1.5% agarose gel in parallel with 100 bp DNA ladder size marker. Figure 1 shows the PCR product amplified from HCV-infected serum.

### Cloning

The PCR product was ligated to PBR322 T-vector (Figure 2) and transformed into *E. coli*. Recombinant plasmid was extracted and digested by SacI and BamHI restriction enzymes. Insert was purified and subcloned in pGemex-1 and named pBKC84 (Figure 3). Figure 4 shows linear recombinant and no recombinant plasmids.

### Gene expression

pBKC84 was transformed in *E. coli* BL21 (DE3) and expression was induced by one mM

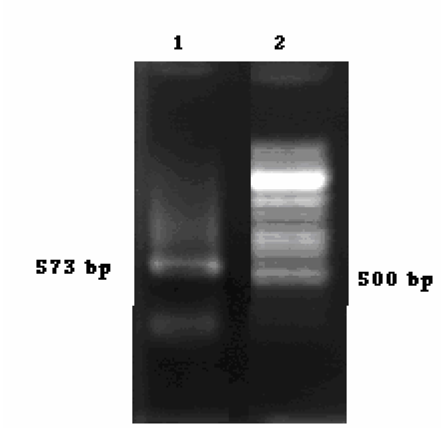


**Figure 1.** 1.5 % agarose gel electrophoresis; lane 1: PCR product of HCV core gene, lane 2: 100 bp DNA ladder marker.

IPTG. Figure 5 shows SDS – polyacrylamide gel electrophoresis loaded by interval sampling before and after induction. The expressed protein is seen on the gel.

### Confirmation of gene expression

Dot blot was used with HCV-positive serum as

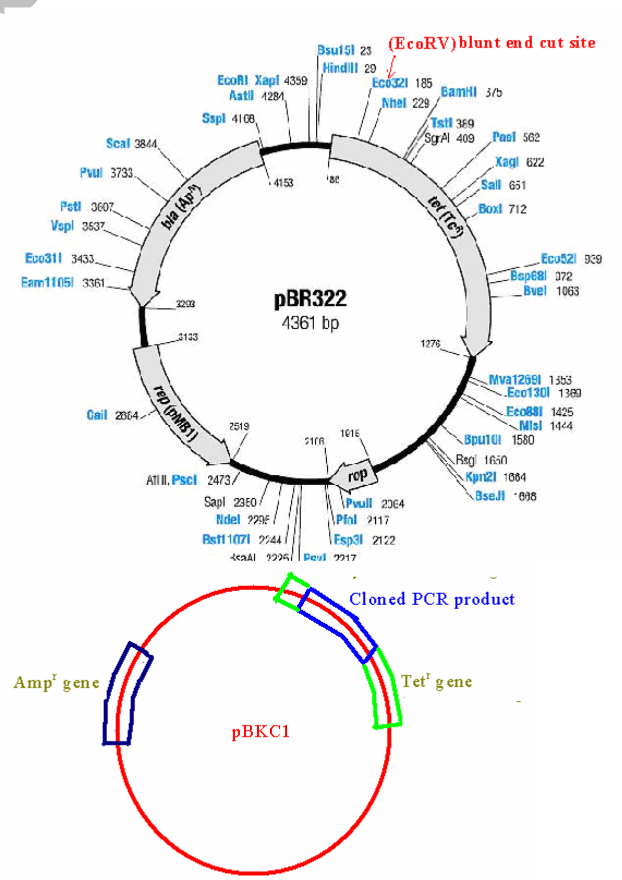


**Figure 2. A)** Map of pBR322 plasmid; **B)** pBKC1 plasmid.

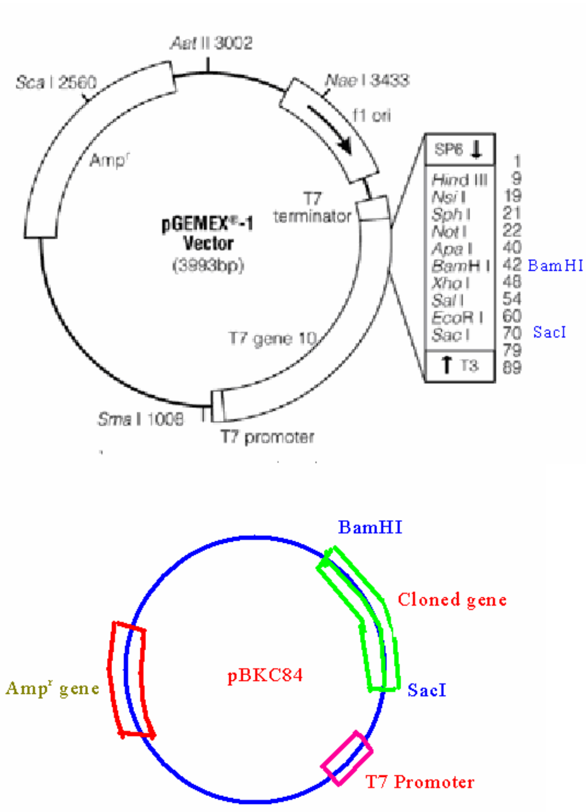


**Figure 3.** A) Map of pGEMEX-1 expression vector; B) pBKC84 plasmid.

antibody and lysate induced cells as antigen. Figure 6 shows the dot blot analysis detected after the enzyme – substrate reaction. Arc produced in gel diffusion by antigen antibody was seen using induced lysate cells as antigen and HCV-infected serum as antibody.

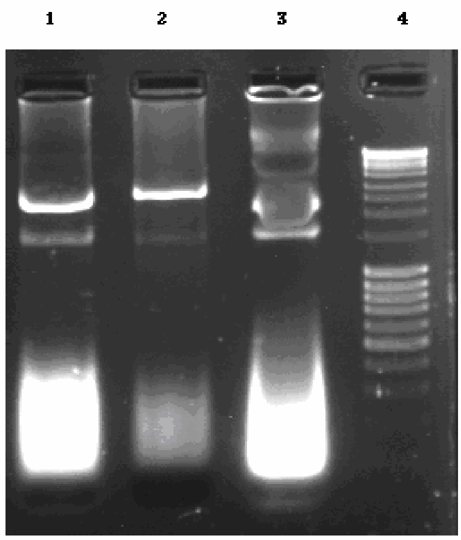


**Figure 4.** 0.8 % agarose gel electrophoresis of plasmids; Lane 1: linear nonrecombinant plasmid, Lane 2: linear recombinant plasmid, Lane 3: circular plasmid, Lane 4: DNA marker.

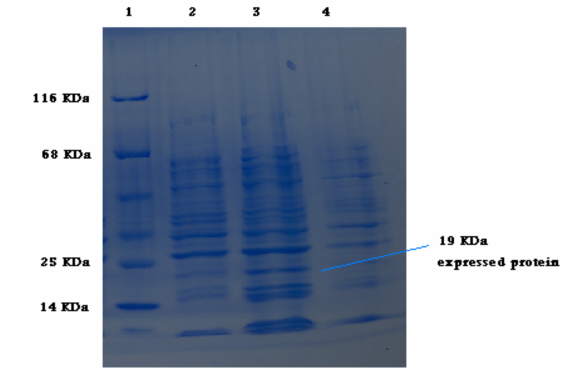


**Figure 5.** SDS- PAGE of induced HCV core gene; Lane 1: protein marker, Lane 2: one hour after induction, Lane 3: three hours after induction, Lane 4: before induction.

## Discussion

Considering the challenges of hepatitis C treatment, it is desirable to develop a vaccine to prevent HCV infection.[2]

HCV core protein appears to be a multifunctional protein that is involved in many viral and cellular processes.[3] Shimoike et al.[7] suggested that the HCV core protein regulates the expression of HCV by interacting with 5` UTR.

Encke et al.[21] used HCV core vaccine and demonstrated that HCV core pulse dendritic cell might serve as a new modality for immunotherapy of HCV especially in chronically infected patients. Gehring et al[22] prepared a DNA vaccine based on HCV core gene which augmented by type 1 interferon and vaccinated mice against HCV. Aguilar et al[23] showed that DNA vaccines, expressing the HCV core gene were able to induce strong immune responses after nasal as well as parenteral administration. They demonstrated that HCV core vaccine enhances the host immune response against hepatitis B surface antigen (HBsAg). Matsui et al.[24] demonstrated that prime double boost immunization involving DNA vaccine based on HCV core gene and replication defective adenovirus expressing HCV core (Adex1SR3ST) can induce core specific cytotoxic T lymphocytes (CTLs). We cloned HCV core protein gene that is one of the most conserved regions of the HCV genome. We present a recombinant protein based on the HCV core gene. The core gene based on GenBank data base contained 573 – 608 nucleotides, but as reported by Duenas-Carrera et al.,[25] its first 176 amino acids are very important and can induce humoral and cellular specific immune responses in humans. Its expression is satisfactory and has the *in vitro*

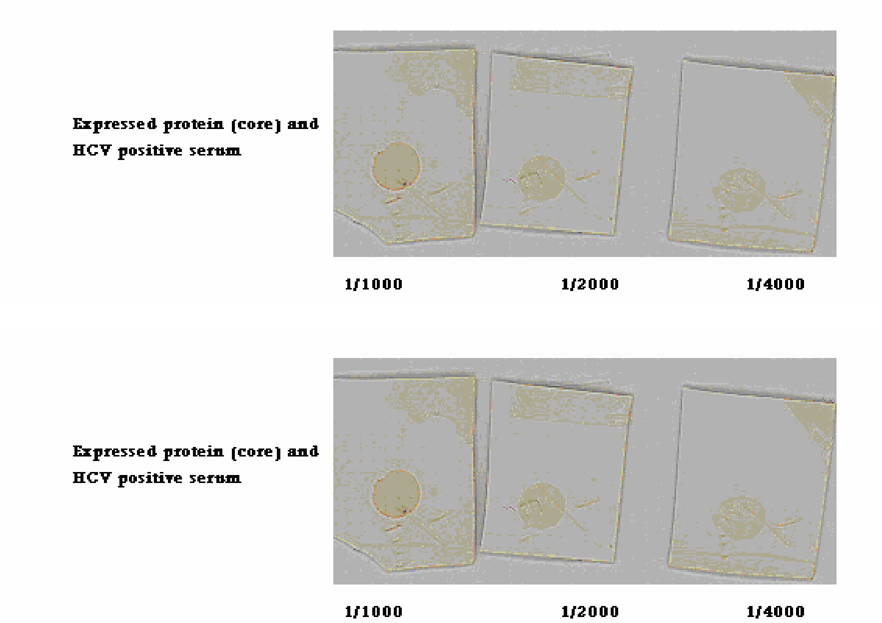


**Figure 6.** Dot blot analysis of recombinant core protein by 1/1000, 1/2000, and 1/4000 diluted HCV -positive serum and detected by human anti-IgG peroxidase conjugated.

biologic effects. The pBKC84 is recomended for use in further studies, both *in vitro* and *in vivo*.

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