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# Construction of high-quality Caco-2 three-frame cDNA library and its application to yeast two-hybrid for the human astrovirus protein–protein interaction

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A B S T R A C T

Human epithelial colorectal adenocarcinoma (Caco-2) cells are widely used as an *in vitro* model of the human small intestinal mucosa. Caco-2 cells are host cells of the human astrovirus (HAstV) and other enteroviruses. High quality cDNA libraries are pertinent resources and critical tools for protein–protein interaction research, but are currently unavailable for Caco-2 cells. To construct a three-open reading frame, full length-expression cDNA library from the Caco-2 cell line for application to HAstV protein–protein interaction screening, total RNA was extracted from Caco-2 cells. The switching mechanism at the 5′ end of the RNA transcript technique was used for cDNA synthesis. Double-stranded cDNA was digested by *Sfi* I and ligated to reconstruct a pGADT7-*Sfi* I three-frame vector. The ligation mixture was transformed into *Escherichia coli* HST08 premium electro cells by electroporation to construct the primary cDNA library. The library capacity was $1.0 \times 10^6$ clones. Gel electrophoresis results indicated that the fragments ranged from 0.5 kb to 4.2 kb. Randomly picked clones show that the recombination rate was 100%. The three-frame primary cDNA library plasmid mixture ($5 \times 10^5$ cfu) was also transformed into *E. coli* HST08 premium electro cells, and all clones were harvested to amplify the cDNA library. To detect the sufficiency of the cDNA library, HAstV capsid protein as bait was screened and tested against the Caco-2 cDNA library by a yeast two-hybrid (Y2H) system. A total of 20 proteins were found to interact with the capsid protein. These results showed that a high-quality three-frame cDNA library from Caco-2 cells was successfully constructed. This library was efficient for the application to the Y2H system, and could be used for future research.



## 1. Introduction

The Caco-2 cell line is a continuous line of heterogeneous human epithelial colorectal adenocarcinoma cells. Caco-2 cells express tight junctions, microvilli, and numerous enzymes and transporters that demonstrate an intestinal absorptive cell phenotype (Chantret et al., 1988).

The use of CaCo-2 as an *in vitro* amplification system for the study of human enteric viruses has been reported in different laboratories worldwide. CaCo-2 cells have shown an increased sensitivity to the laboratory strains of group A rotavirus (Wang et al., 2012), reovirus (Tyler et al., 2001), norovirus (Duizer et al., 2004), astrovirus (Jang et al., 2010), enterovirus 70 (Pinto et al., 1994), poliovirus (Ammendolia et al., 1999), echovirus (Reigel, 1985), and adenovirus (Zhu et al., 2004). The wild-type infectious rotavirus, astrovirus, and adenovirus in the stool samples of patients with acute gastroenteritis can infect CaCo-2 cells (Willcocks et al., 1990; Dickman et al., 2000; Sherwood et al., 2012).

Information on viral replication and their interactions with host organisms is required for developing strategies to prevent virus infection. The yeast two-hybrid (Y2H) screens of the human cDNA library have been used to study viral interactions with the host cell factors (Rajagopala et al., 2012). To screen the virus and Caco-2 cell interaction protein, an efficient and high-quality full-length cDNA library is necessary for studying the protein function and finding the protein associated with the defense mechanism against viral infections.

This study described a method for constructing a full-length three-frame cDNA library of Caco-2 cells using the switching mechanism at the 5′-end of RNA transcript (SMART) technology. Moreover, the study also determined the efficiency of the cDNA

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**Fig. 1.** Reconstruction of pGADT7-*Sfi* I vector for the generation of three reading frames. pGADT7 vector was reconstructed to represent the three possible reading frames by inserting one (A) or two nucleotides (AA) behind the second ATG of a pGADT7 vector. The underlined site is the Restriction Enzyme site *Sfi* I; 5′ end contains *Sfi* IA restriction sites; and 3′ end contains *Sfi* IB restriction sites; the downward arrow is the inserted nucleotide.

library in screening the human astrovirus (HAstV) capsid protein interaction using Y2H technology.

## 2. Materials and methods

### 2.1. Cell culture

Caco-2 cell line was obtained from Cell Resource Center, IBMS, and CAMS/PUMC (Beijing China). Caco-2 cells were grown in six-well plates (Nunc, Roskilde, Denmark) with Dulbecco's modified eagle's medium supplemented with 10% fetal bovine serum. The passage of cells was 32th.

### 2.2. cDNA library primer

Universal primers were designed to amplify the inserted fragments of the cDNA library according to vector pGADT7 sequence, and synthesized by Shanghai Sangon Company (Shanghai, China) as follows: F primer: 5′-GGAGTACCCATACGACGTACC-3′; R primer: 5'-TATCTACGATTCATCTGCAGC-3'.

### 2.3. Total RNA isolation

The total RNA from Caco-2 cells (passage 32th) was isolated using Trizol (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions, and treated with DNase I to remove genomic DNA. The integrity of total RNA was analyzed by 1% agarose gel electrophoresis. The concentration and purity of total RNA were determined by a spectrophotometer (Eppendorf AG, Hamburg, Germany) at 260 and 280 nm, respectively.

### 2.4. pGADT7-Sfi I vector reconstruction

pGADT7 vector was reconstructed to represent the three possible reading frames by inserting one nucleotide (A) or two nucleotides (AA) behind the second ATG of the pGADT7 vector. The directional cloning site was *Sfi* I A [(gene 5′): GGCCATTACGGCC] and *Sfi* I B [(gene 3′): GGCCGCCTCGGCC]. cDNA inserted into the *Sfi* I of pGADT7 was expressed in three reading frames (frames 1, 2, and 3) (Fig. 1).

### 2.5. cDNA synthesis

The single-stranded cDNAs (sscDNAs) were synthesized using a SMART™ cDNA Library Construction Kit (Clontech USA, Mountain View, CA) according to the manufacturer's instructions. sscDNAs were synthesized by 1000 ng of total RNA using SMART IV oligonucleotide (5′-AAGCAGTGGTATCAACGCAGAGTGGCC-ATTACGGCCGGG-3′), CDS III/3 primer (5′-ATTCTAGAGGCCGAGGCG GCC GACATG-d(T)30VN-3′), and SMARTScribe Moloney murine leukemia virus (MMLV) Reverse Transcriptase. Double-stranded cDNAs (dscDNAs) were acquired by 20 cycles of long-distance PCR using Advantage 2 Polymerase Mix (Clontech USA, Mountain View, CA) with 2 μL of sscDNA product. The reaction parameters were as follows: denaturation at 95 °C for 1 min, followed by 20 cycles of 95 °C for 15 s, and 68 °C for 6 min. The PCR products (5 μL) were analyzed on 1% agarose/ethidium bromide (EtBr) gel. The PCR products of dscDNAs were purified by a Chroma Spin TE-400 column (Clontech USA, Mountain View, CA) to remove low-molecular-weight cDNA fragments, small DNA contaminants, and unincorporated nucleotides from cDNA. The purified dscDNAs were quantified by electrophoresis on 1% agarose/EtBr gel.

### 2.6. cDNA three-frame primary library construction and evaluation

The purified dscDNAs were ligated into a pGADT7-*Sfi* I vector (Clontech USA, Mountain View, CA) with T4 DNA ligase (Takara Bio, Otsu, Japan). The constructs were then electro-transformed into *Escherichia coli* HST08 premium electro cells (Takara Bio, Otsu, Japan). The titer and reorganization ratio of the library were calculated by counting the quantity of leukoplakia and locus coeruleus colonies as follows: titer of cDNA library (pfu/mL) = total colony number × dilution multiple × $10^3$/volume of the connection product; reorganization ratio = leukoplakia quantities/total colony number × 100%.

Sixteen colonies were randomly selected to identify the size of the inserting cDNA fragment using F and R primers based on the following PCR conditions: 94 °C for 5 min; 30 cycles of 94 °C for 50 s, 68 °C for 50 s, 72 °C for 1 min; and 72 °C for 10 min. The PCR products (5 μL) were analyzed using 1% agarose/EtBr gel.

### 2.7. Three-frame cDNA library amplification

The constructed three-frame cDNA library plasmids (frames 1, 2, and 3) were mixed and electro-transformed into *E. coli* HST08 premium electro cells (Takara Bio, Otsu, Japan). All clones were harvested and added to 400 mL of SOC medium with 50 μg/mL ampicillin solution by constant shaking (200 rpm to 250 rpm) at 37 °C overnight ($OD_{600}$ = 6). The amplified library plasmid was extracted using a NucleoBond Xtra Maxi EF kit (Macherey-Nagel).