STUDY ON CONSTRUCTION OF cDNA LIBRARY OF THE TREATED CHANGLIVER CELL AND QUALITY ANALYSIS

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ABSTRACT

The study aims to construct cDNA library of Changliver cell by SMART (switching mechanism at 5' end of RNA transcript) technique and analyze its quality. cDNA of Changliver cell was made with RT-PCR and LD-PCR (long-distance PCR), the cDNA library was constructed with SMART cDNA library construction kit. Through testing, the high quality cDNA library containing whole long cDNA of Changliver cell had been constructed. The titer of the amplified cDNA library was 4.5×10^{10} pfu/ml and the average exogenous inserts of the recombinants is 1.5 kb. These results showed that the Changliver cell cDNA library had an excellent quality and lay foundation for screening whole long cDNA of related genes.

KEY WORDS

cDNA library; SMART; Changliver cell; LD-PCR; immunoscreening

Changliver cell was a cell stain coming from human liver cancer tissue, which was often used in scientific research. Study revealed, there were many new proteins existing, when Changliver cell was treated with DM (dedifferentiation medium) then recovered for 24 hours, relative to the normal (1). For studying the function of these new proteins, it was necessary to clone the whole long cDNA. Some methods, by which the whole long cDNA could be made, were compared, for example RACE (rapid amplification of cDNA end), screening of cDNA library (2). We found it was more effective to construct cDNA library, and screen objective genes by antibody or nucleic acid probe. So cDNA library of Changliver cell treated by DM and recovered for 24 hours was constructed. Through analysis, the cDNA library made with high fidelity. The cDNA library was screened with common antibody of ADAMs (a disintergrin and metalloproteinases) composed of conservative region of several ADAMs molecules known, and

several new genes or ADAMs relative genes were mixed. Here, it was introduced how to construct the cDNA library and analyze its quality.

MATERIALS AND METHODS

Preparation of Changliver cell

Changliver cell was cultured in CO₂ incubator, 37°C, 5.0% CO₂. The culturing cell was treated by DM for 30 minutes, then allowed for 24 hours in normal medium, 37°C, 5.0% CO₂. The treated cell was collected by centrifugation.

Extraction of total RNA

Isolation of total RNA was carried out according to the protocol of RNAase kit (QIAGEN). The quantity and integrity of total RNA was detected by ultraviolet spectrometer and by electro-phoresing on a denaturing formaldehyde agarose gel cutter with EtBr (ethidium bromide) (3).

Primers of constructing cDNA library

Oligonucleotide: 5'-AAGCAGTGGTATCAACGC AGAGTGGCCATTACGGCCGGG-3'; CDS□ / 3'PCR primer: 5'-ATTCTAGAGGCCGAGGC GGCCGACATG-d(T)³⁰N-3'; 5'PCR primer: 5'-AAGCAGTGGTATCGGCGCAGAGT-3'.

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Generating a cDNA library

According to the protocol of SMART cDNA library construction kit (Clontech) and package protein of λ phage (Stratagene), first strand cDNA was composed using reverse transcriptase by RT-PCR. Second, double strands cDNA was constructed with first strand cDNA by LD-PCR (long distance PCR). Third, the product of LD-PCR was digested by proteinase K, then cDNA was extracted by phenol/chloroform and precipitated by ethanol and NaAc (4). Fourth, cDNA was digested by *Sfi*I for generating cohesive ends to link to λ phage. Fifth, the fragments were selected through Chroma Spin-400 column (Clontech) to 16 Ep tubes, then 1.1% agarose gel electrophoresis was prepared to test the product selected, and the liquid of three lightest tubes was collected and precipitated; Sixth, the cDNA digested by *Sfi*I was linked to two arms of λ phage, then packed with package protein of λ phage. The unamplification cDNA library was constructed.

Quality analysis of cDNA library

To calculate the titer of unamplification library, if the number of phage was less than $1\sim 2 \times 10^6$ pfu/ml, the linking was repeated again. After calculating the recombination fraction, the cDNA library was amplified, then the titer of the amplification library was calculated. A successful cDNA library amplified should contain to about 10^{10} pfu/ml. $\text{Pfu/ml} = (\text{number of plaques} \times \text{dilution factor} \times 10^3 \mu\text{l/ml}) / (\mu\text{l of diluted phage plated})$.

RESULTS

The quantity and integrity of total RNA

After total RNA was extracted, to measure the concentration by diluting 10 μl of total RNA into 1 ml of TE (1:100 dilution) and measure absorbance at 260 nm and 280 nm. The OD at 260 nm and 280 nm was about 1.9 (A_{260}/A_{280}). 0.8% denaturing formaldehyde agarose gel electrophoresis showed clear 28S rRNA and 18S rRNA, and the brightness rate of 28S rRNA and 18S rRNA (28S/18S) was 2:1 (Fig. 1).

Construction of cDNA library

After linked to λ phage *in vitro*, packed and infected host bacteria, the titer of unamplified library was 2.2×10^7 pfu/ml, and the titer of amplified library arrived to 4.5×10^{10} pfu/ml. The results are in good agreement with constructing library. The recombination fraction was analyzed by X-gal, and there were 8 blue plaques and 1360 colorless plaques in one plate, so the recombination fraction was $1360/(1360 + 8) \times 100\% = 99.5\%$.

Fig. 1 Denatured agarose gel electrophoresis of total RNA in Changliver cell

PCR analysis of cDNA length in library

We randomly selected 50 clones to isolate plasmid and performed PCR with CDS $\square$ /3'PCR primer and 5'PCR primer. Results showed that all clones contained insert fragments between 200 bp and 4000 bp, the average exogenous inserts of the recombinants was 1.5 kb (Fig. 2).

Fig. 2. Random amplification of cDNA in library by PCR

DISCUSSION

Holding common antibody of the known proteins, how to get the whole length cDNA, RT-PCR, RACE and immunoscreening of cDNA library were often done (5). But it is more difficult to clone the whole cDNA by RT-PCR or RACE since unreal positive and needy fragment join each other (6). Constructing and screening cDNA library is a more effective method to catch the whole length cDNA of new protein using normal antibody by immunoscreening (7). So the cDNA library of Changliver cell was constructed.

The cDNA library constructed by old method has one expressing model. If the incompleteness of transcription happen, the correct expressing product could not appear and difficult to immunoscreening (8). The SMART cDNA library construction kit we

used is a new product of Clontech company. The special design can make three reading frames of each insert which on sequencing did not lose any information (9). The other advantage of the cDNA library we constructed also contain whole long cDNA. Normally, when mRNA was reversely transcribed into first strand cDNA, the transcription had already stopped factually before mRNA transcription was thoroughly over (10). But in this study, by using SMART (switching mechanism at 5' end of RNA transcript) technique, the whole long mRNA could be thoroughly transcribed into cDNA.

The quantity and quality of cDNA is a key to construct high quality cDNA library (11), but the quality of cDNA is affected by mRNA, so the total RNA was isolated by RNAase kit (Qiagen) in our study, and on comparison, we thought that more efficacious than other methods to extract RNA (3). The quantity and integrity of total RNA detected by ultraviolet spectrometer and by electrophoresing on a denaturing formaldehyde agarose gel was good. When performing LD-PCR, the same temperature was applied during annealing and extention, and the time of extention was delayed for obtaining integrated cDNA (12). We had screened the cDNA library using common antibody of ADAMs and identified several new genes (the result was reported in another paper).

Thus, the high quality cDNA library of the treated Changliver cell has been constructed, which made better base to study Changliver cell and in order to identify gene in Changliver cell.

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