Construction and Characterization of a cDNA Expression Library from the Endangered *Jinnan* Cattle

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Abstract: Jinnan cattle is one of the most important species in China, It is also listed as one of the 78 nationally protected domestic animals by the Chinese government in the year of 2000. The construction of cDNA expression library of Jinnan cattle is of great significance for its protection of genetic resources, and it is very important for the research of gene function. In this study, the total RNA was extracted from the ear tissue of Jinnan cattle, then the ear tissue cDNA expression library of Jinnan cattle was constructed using SMART^{TM} technique. The result showed that the titer of amplified cDNA library is 1.17×10^{10}pfu·mL^{-1}, the rate of recombinant is above 93.47%, and the average size of the fragments is 0.7 kb. This study has an important significance for the preservation of Jinnan cattle gene resources.

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1. Introduction

Jinnan cattle is one of the most important species in China, it was listed as one of the 78 nationally protected domestic animals by the Chinese government in the year of 2000. Constructing Jinnan cattle cDNA library for the protection of genetic resources, as well as the study of gene function has an important genetic significance (Shurong Zhao, 2008).

cDNA library refers to a biological developmental stages of a transcription of all mRNA, by reverse transcription of cDNA fragments, a vector could be formed by connecting a collection of clones (Qingsheng Wang, 2009). The establishment and characterization of Chinese Jinnan cattle cDNA library, our aim is not only to preserve this nationally protected breed resource, but also provide molecular markers linkage map of the building used by probes, more importantly, it could be used to separate full-length genes and then to carry out gene function research (Ruffini, 2007).

2. Material and Methods

Samples were taken from the South of Shanxi Yuncheng city, Shanxi Province, Linyi County Cattle conservation farm, ear marginal tissues were obtained by ear clamp, then they were immersed in ice-box with RNA Locker and transported to the laboratory, stored in -80 ℃ refrigerator.

Ear marginal tissue samples were cut into small pieces (100mg), placed into liquid nitrogen quickly. Repeatedly frozen, stored at -80 ℃ or directly extracted RNA, 100mg frozen tissue was put into a mortar filled with liquid nitrogen, crushed with pestle research organization, then the powder was moved into a centrifuge tube containing 1 ml Trizol reagent, put it aside at room temperature after mixing 5 min, adding 0.2 ml chloroform, and vortex mixing the oscillator oscillation 15 s at room temperature, put it aside for 2-3 min, 4 ℃ 12000 r/min for 15min, take the upper aqueous phase to another centrifuge tube, add 400 μl isopropanol, mixing at room temperature then put it aside after 10 min, 4 ℃ 12000 r/min for 10min, washed with 1 ml 75% ethanol, precipitated twice, 37℃ 5-10 min to dry ethanol, finally, the total RNA was dissolved in 50-100 μl DEPC-treated water.

Synthesis of cDNA first strand was according to clontech's SMART^{TM} cDNA library construction Kit, adding the following samples in a sterile 0.5 ml centrifuge tube: 3 μl total RNA sample, 1 μl SMART III Oligonucleotide, 1 μl CDS III 3 `PCR Primer, deionized water, make up 5 μl. 72 ℃ 2 min, ice-cooled 2 min, To take another test tube by adding the following reagents: 2.0 μl 5 × First-Strand buffer, 1.0 μl DTT (20mM), 1.0 μl dNTP mix (10mM), 1.0 μl MMLV reverse transcriptase (200U/μl), the total volume was 10.0 μl. 42 ℃ incubated for 1 hr, on ice to terminate reaction.

Take 2 μl first strand cDNA product for the LD-PCR amplification of cDNA, adding the following samples in a sterile a PCR tube: 2 μl first strand cDNA, 80 μl deionized water, 10 μl 10 × advantage 2 PCR Buffer, 2 μl 50 × dNTP Mix, 2 μl 5 `PCR Primer, 2 μl CDS III / 3 `PCR Primer, 2 μl 50 × advantage 2 Polymerase Mix, the total volume was

100μl. PCR reaction was according to the following amplification program: 95 ℃ 25 sec, 95 ℃ 25 sec, 68 ℃ 6 min, 21 cycles.

After proteinase k digestion, chroma SPIN-400 column separation, connection, λ phage packaging, Picked from the plate work VCS257 monoclonal inoculated 15 ml LB/MgSO4/maltose liquid medium in vitro. 37 ℃, 140 r/min overnight train, until the OD600 to 2.0, 5,000 r/min centrifuge 5min, abandoned on the clear liquid, precipitation with 7.5 ml 10 mM MgSO4 suspension, ready enough to 100 mm LB/MgSO4 flat, preheating, prepare 5 ml test tube, add 500μl of the overnight bacteria and sufficient to form 6-7 × 10^4 phage clones dilution packaging samples, 37 ℃ water bath-15min. Each tube plus 4-5 ml melt LB/MgSO4 soft top agarose, rapid mixing shop to LB/MgSO4 flat, cooling plate at the proper temperature 10 minutes, so that the top agarose hardening. Inverted plate at 37 ℃ for 6-18 hours, until plaque contact with each other. Each plate plus 12 ml 1 × lambda dilution buffer, 4 ℃ overnight. Flat in the horizontal shaking the bed in order to train 50r/min proper temperature 1 hr, the λ phage lysis buffer into the sterile beaker to obtain an integrated amplified library lysate.

Monoclonal plate was inoculated into 20 ml LB/MgSO4/maltose (plus appropriate antibiotics) liquid medium in vitro.37 ℃, 140 rpm overnight train, until the OD600 to 2.0. 5,000 rpm for 5min. Supernatant was precipitated with 7.5 ml 10 mM MgSO4 suspension. the library with 1 × lambda dilution buffer diluted 1:10000 and SM buffer. 37 ℃ water bath, 15min, each tube by adding 3 ml molten (45 ℃) of the LB/MgSO4 top agarose, rapid reversal of mixing, immediately to the shop has 37 ℃ preheating the LB/MgSO4 plate and quickly absorbed shop will be flatcool 10 minutes in the proper temperature, so that the top agar hardened, inverted plate at 37 ℃ for at least 6-7 hours culture. Calculate the titer (pfu / ml) pfu / ml = (number of phage plaques × dilution factor × 10^3) / μl (diluted phage decking).

3. Results

Take appropriate Jinnan Cattle ear marginal tissue extract total RNA, denatured by formaldehyde agarose gel electrophoresis can clearly see that 28 S and 18 S 2 bright bands, and the 28 S and 18 S ratio of 2 brightness: 1, UV spectrophotometer measured the concentration of 0.83ug/μL, A260/A280 ratio was 1.93, indicating, the total RNA extracted from non-degradable and there are no other substances, pollution has reached the experimental requirements (Figure 1a).

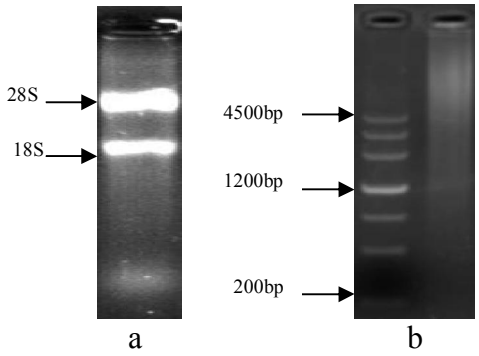

Figure 1. a, Jinna Cattle ear marginal total RNA formaldehyde denaturing agarose gel electrophoresis
b, LD-PCR amplification products by agarose gel electrophoresis

The use of SMART technology, the first strand reverse transcription by LD-PCR reaction, synthetic double-stranded cDNA. By 1.1% agarose gel electrophoresis, band was dispersed, mainly in the 300bp-4kb (Figure 1b), in line with the requirement of experiment.

The double-stranded cDNA Sfi Ⅰ digestion by Chroma SPIN-400 column fractionation large cDNA fragments, electrophoresis results showed that the first 6,7,8 and 9-eluting material is greater than the length cDNA of 300bp (Figure 2a).

Test results showed that the titer of unamplified cDNA library was 1.93×10^6 pfu/mL, the titer of amplified library reached 1.17× 10^{10} pfu/mL. 96 randomly picked phage clones for monoclonal PCR, by 1.0% agarose gel electrophoresis results showed that six empty (including the fragment length of less than 300bp), the positive of 90, re-rate was 93.47%, the average length of the inserted fragment was about 0.7kb (Figure 2b).

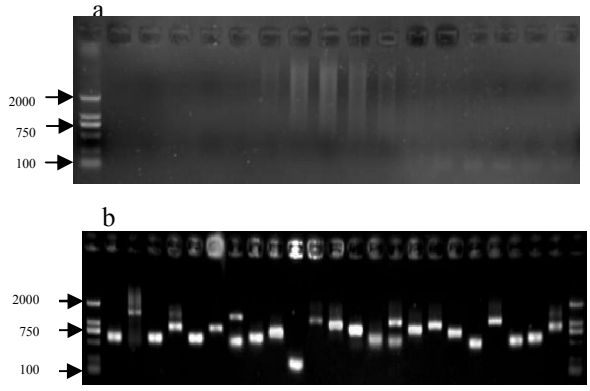

Figure 2. a, Synthesis of Jinnan cattle ear marginal double-stranded DNA before column separation
b, cDNA insert size by PCR detection

4. Discussions

By RNA formaldehyde denaturing agarose gel electrophoresis, it was in order to test the band does or not appear as a standard. As the total RNA was 90% of rRNA, so that the electrophoresis pattern of rRNA, including the 28S, 18S and 5S rRNA. mRNA was dispersed during the period. 1.0% formaldehyde denaturing agarose gel electrophoresis of total RNA quality, 28S and 18S bands were clear, the brightness was about 2:1, UV-spectrophotometer A260/A280 ratio was 1.93, the concentration was 0.83 ug/μL, it was showed that the total RNA was high quality, RNA with no degradation of integrity is better. In addition, there is no additional sample ran out of holes in the DNA, which shows that total RNA samples were without DNA contamination. Total RNA were obtained on the proposed LD-PCR, the results are needed fragments, and make the connection, transformation, etc., thus obtaining the total that the proposed RNA quality is relatively high, it could be used for further library construction (James, 1991).

Evaluation of the quality of cDNA library from the library and re-rate the capacity of two ways (Maria, 2005). Construction of cDNA library aims to find more meaningful cDNA cloning method using PCR amplified from the library promptly gene, the library must be as much as possible, including all mRNA molecules reverse fragment, so that each gene has the opportunity to be cloned. Both in terms of capacity or the library insert sizes respects in accordance with quality requirements, library has a certain integrity of representation and sequence, can be used for further research. As a result of Clontech's SMART^{TM} technology, on the Joint primer and reverse transcriptase are optimized, they can effectively remove the non-poly A tail of RNA and genomic DNA, the reverse transcription for the mRNA 5 'end of time , Super Script II reverse transcriptase as a "template jump" function will be a specific SMART IV oligonucleotide connector to the mRNA 5 'end, reverse transcription enzyme jump shift and continue to the end of the connector, as such jumps often occur in eukaryotes cap structure, only contains the full mRNA and SMART II oligonucleotide template joints in the LD-PCR reaction to amplify them, thus simplifying the RNA purification process to ensure high quality full-length cDNA of access, increased length cDNA library contained in the ratio.

According to the Clontech gene library of the company on good quality standard: The original library of recombinant number of 5×10^5-5×10^8, more than 90% re-insert cDNA fragment is not less than 0.3kb, we constructed cDNA library did not expand by library titer 1.93×10^6 pfu/mL, the titer of amplified library was 1.17×10^{10} pfu/mL, recombination rate was 93.47 percent, reaching library construction requirements.

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