Construction of cDNA library in *Epimedium brevicornum* leaves

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Abstract: The total RNA was extracted from young leaves of *Epimedium brevicornum*, and mRNA was purified by oligo (dT)-cellulose column chromatography, which was reverse transcripted into cDNA using SMART (the Switch Mechanism At the 5' end of RNA Templates) technique. The resulting cDNA was digested by Sfi, the digested fragments were fractionated by CHROMA SPIN-400, and then ligated to λTriplEx2 vector, the mixture of ligation was finally packed into Lambda virons with packaging extracts. 6×10^5 recombinants had been obtained. 80 percent of products were over 500bp. 20 clones were chosen randomly from the library and were screened cDNA inserts using PCR. The results showed that the cDNA library of young leaves of *E. brevicornum* had been constructed, and it could be used to screening to isolate particular clones.

Key words: *Epimedium brevicornum*; young leaf; cDNA library

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Epimedium brevicornum, Yin Yang-Huo in Chinese, is traditional Chinese herbal medicine, the aerial parts of *E. brevicornum* is listed as source plants in Chinese pharmacopoeia (the state Pharmacopoeia commission of PRC, 2000). "Yin Yang-Huo" was frequently used for treating senile functional disease and enhancing kidney function, it can also strengthen physique, cure rheumatism. In recent years, domestic researchers have done further work on *E. brevicornum* and have made great progress in physiological study, especially the study of the flavonoids the important class of secondary products which are widely distributed inspermatophytic plants and have a variety of roles in plants, animals and micro-organisms (Andrea *et al.*, 2002). Although the study of the flavonoids of the *E. brevicornum* is abundant, it is mainly focus on the study on the effect of physiology, and no study touch upon to the molecular of the flavonoids.

Numerous flavonoids accumulate abundantly in *E. brevicornum*, it play great role in protecting the plant from UV, provide pigmentation to attract pollinators, and it also act as antibiotics in plant defense responses (Kubasek *et al.*, 1992; Koes *et al.*, 1994; Shirley, 1996). Individual plant species can synthesize a variety of flavonoid compounds. "Yin Yang-Huo" species are of much interest because they accumulate a large quantity of flavonoids, especially in Yin Yang-Huo's green tissues and flowers. The biosynthetic pathway for the flavonoids were well established (Schram *et al.*, 1984; Wiering *et al.*, 1984), and these flavonoids must be catalyzed by many key enzymes such as chalcone synthase (chs), flavanone-3-hydroxylase (f3h) and flavonol synthase (fls) etc. before they play the function.

A cDNA library is a collection of different DNA se-

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quences from an organism each of which has been cloned into a vector for ease of purification, storage and analysis (Turner *et al.*, 2003). In order to investigate the key enzymes in the flavonoids biosynthesis pathway of the *E. brevicornum*, the cDNA library of *E. brevicornum*'s young leaf was constructed in this experiment.

1 Materials and Methods

1.1 Plant materials

Young leaves of *E. brevicornum* were collected and dehydrated in liquid nitrogen, then stored at -80 ℃ for use.

1.2 Isolation of total RNA and mRNA

Total RNAs of young leaves were extracted by the method of Trizol, and then stored in -20 ℃ after purified, and then analyzed by gel eletrophoresis and UV spectrophotometer. Poly(A) RNA was purified by oligo(dT)-cellulose column chromatography according to the manual of Pharmacia mRNA purification kit. After washing with 0.5 mmol/L NaCl, 0.1 mmol/L NaCl, the mRNA was collected with TE buffer, and the concentration of the mRNA was measured through an UV spectrophotometer.

1.3 cDNA synthesis and library construction

The sscDNA and dscDNA synthesis and library construction were carried out according to the manual of SMART™ cDNA Library Construction Kit (Clontech). About 2 μg mRNA was reverse transcribed to single stranded cDNA by Superscript Ⅱ (Gibco BRL) at 42 ℃ for 1 h. One fifth of the first-strand cDNA was used to carry out dscDNA synthesis by a 22 cycles of PCR(95 ℃ for 15 s, 68 ℃ for 6 min). All of the dscDNA were digested with sfi. The reaction product was fractionated by CHROMA SPIN-400 and the fractions containing dscDNA larger than 500bp were collected, and re-dissolved in dd H_2O after deposited in alcohol. And at last, these dscDNAs were ligated into lambda TripIEx 2 vector.

1.4 Tittering the unanplified library and determining the percentage of recombinant clones

A single, isolated colony from the streak plate was picked out randomly, which was used to tittering the library according to the manual of SMART™ cDNA Library Construction Kit, and in order to test for ligation efficiency, 20 negative single clones were picked out randomly used to PCR, and then checked by gel eletrophoresis.

2 Results and Discussing

The *E. brevicornum*'s young leaves were collected in early January. It was not only because flavonoids were expressed maximally, but also many valuable genes were expressed in this period. From 30 g young leaves, about 150 μg total RNA was obtained. The electrophoresis analysis showed that there were apparent integrate bands of 28S and 18S rRNA, and the 28S band exhibited nearly about twice of the 18S (Fig. 1). From UV photometric measurement, it could be seen that A_{260} nm/A_{280} nm=1.82. These characters demonstrated a high quality of the total RNA. 2.0 μg mRNA could be obtained after Oligo (dT)-cellulose column chromatography from 150 μg total RNA.

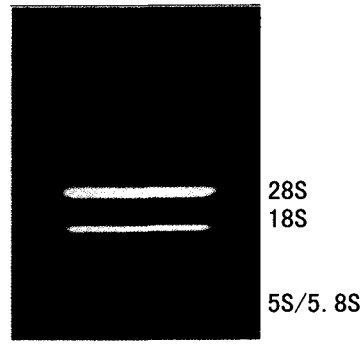

Fig. 1　Total RNA of the *Epimedium brevicornum*'s young leaves

A modified oligo (dT) primer (CDS Ⅲ/3' PCR primer) primed the first-strand synthesis reaction by use of 2 μg mRNA. One fifth of the resulting sscDNA was amplified by PCR using CDS Ⅲ/3' PCR primer and 5' PCR primer derived from SMART Ⅲ oligonucleotide and obtained the dscDNA. The products were checked by gel electrophoresis, the result showed that it produced a dispersive molecules bands from about 0.5 kb to 4.5 kb (Fig. 2), it showed that the quantity of the dscDNA was enough. Comparing with other ones, a higher percentage

of full-length cDNA could be obtained with this method (Li *et al.*, 2004; Bai *et al.*, 2002).

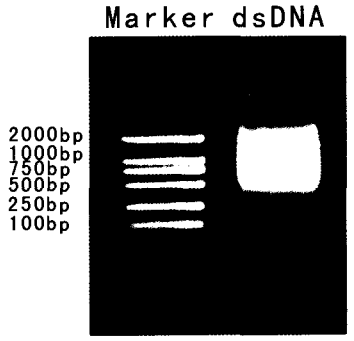

Fig. 2 dscDNA synthesis by a 22 cycles of PCR

The dscDNA was digested by sfi, and the resulting of cDNAsize fractionation by CHROMA APIN-400, 17 tubes of different sizes of fragments were separated. And then checked by gel electrophoresis (Fig. 3), the cDAN in 7～10th tube were collected and combinated together, here, the cDNA in 7th tube was also be collected although it was faint in order to avoid losing the big fragment because of the lower resolving power of the gelose. The 11th and 12th tube were not be collected because the fragments of them were small.

The collected cDNA was deposited in alcohol, and then re-dissolve in 7 μL dd H_2O. 0.5 μL dissolved cDNA was compared with the control cDNA, the concentration of the dissolved cDNA was about 68 ng/μL after the UV photometric measurement (Fig. 4), so the total cDNA will be about 900 ng. The quantity of the sample cDNA could be used to construct the cDNA library.

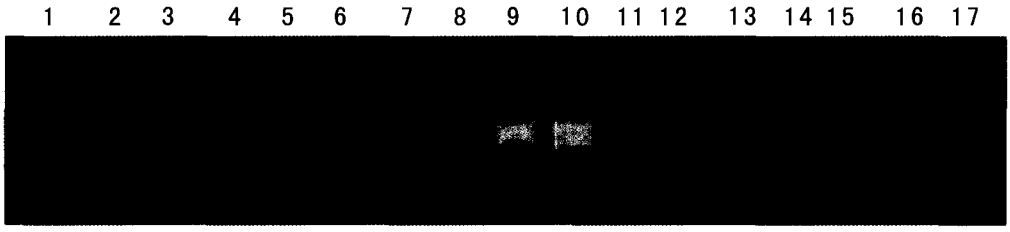

Fig. 3 The dscDNA after size fractionation by CHROMAAPIN-400

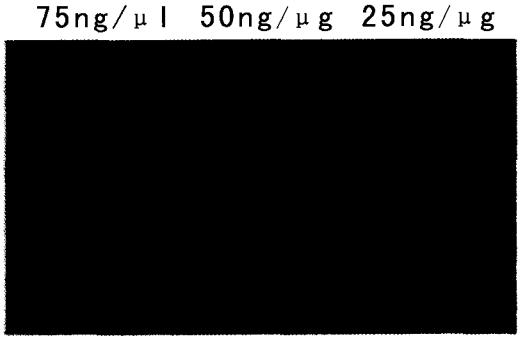

Fig. 4 Detection of the concentration of the purified cDNA
The lower one is the sample cDNA, and the upper are the control cDNA

The eligible dscDNA was ligated to λTriplEx2 vector, here, the ratio of cDNA to vector in the ligation reaction was a critical factor in determining transformation efficiency, and ultimately the number of independent clones in the library. And the optimal ratio of cDNA to vector in ligation reactions must be determined empirically for each vector/cDNA combination. According to the manual of SMART™ cDNA Library Construction Kit, the dscDAN was successfully ligated to the λTriplEx2 vector, and then packed it into Lambda virons with packaging extracts finally. The titer of the cDNA library was 1.2×10^6 Pfu/mL. In order to test the efficiency of the ligation, randomly 20 negative single clones were picked out used to PCR after the cDNA was cut in

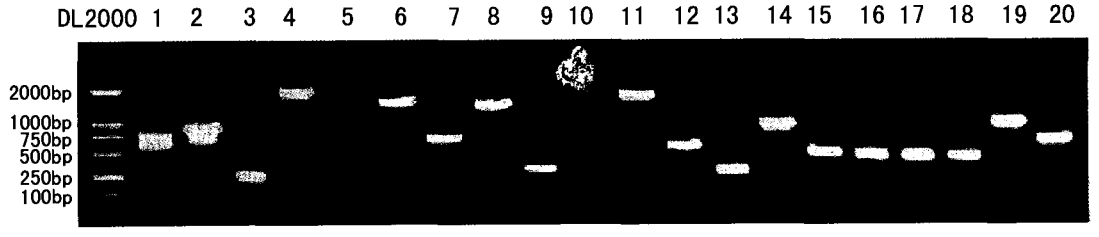

Fig. 5 Detection of inserts of the library and the combining rate

intro, the sizes of the products of PCR were mainly 0.5 ~2 kb(Fig. 5), the average size of the fragments is about 975 bp, efficient ligation of the cDNA to the λTriplEx2 vector result in 80% recombinants.

E. brevicornum's young leaf cDNA library was constructed successfully in this experiment. The quantity and the quality of the total RNA and the mRNA were very important in constructing the cDNA library, and the presence of the molecular weight material in the size-fractionated dscDNA must be over 0.4 kb. And when chose the vector, the λTriplEx2 vector was well be chosen to use, this vector allow digestion with multiple enzymes which make the stuffer fragment unclonable, and it also provides other advantages, such as high titer libraries, blue/white screening for recombinants, regulated expression of cloned inserts, and every cDNA inserted into the MCS of λTriplEx2 is expressed in all three reading frames.

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淫羊藿嫩叶 cDNA 文库的构建

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摘　要：以淫羊藿嫩叶为实验材料，用 Trizol 方法提取植物总 RNA，纯化出 mRNA，用 SMART(the Switch Mechanism At the 5′end of RNA Templates)技术反转录成 cDNA，同时使用 CHROMA SPIN-400 凝胶柱层析纯化 cDNA，最后将片断连入 λTriplEx2 vector，经包装得到 500 μL 原始文库，文库的滴度为 1.2×10^6 Pfu/mL。经体内切割后，随机挑选文库的 20 个阳性克隆进行 PCR 鉴定，算出文库的重组率为 80%，扩增出的片断主要集中在 0.5～2 kb 之间。结果说明文库质量较好，可以用于基因筛选。

关键词：淫羊藿；嫩叶；cDNA 文库