一步 PCR 快速扩增辽宁碱蓬甜菜碱醛脱氢酶 cDNA 3′末端序列

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摘 要:根据已获得的辽宁碱蓬甜菜碱醛脱氢酶 cDNA 的部分序列,设计一条基因特异性引物,与通用引物并用,一步 PCR 成功地克隆了辽宁碱蓬甜菜碱醛脱氢酶 cDNA 3′末端。与常规的 3′RACE 法相比,一步 PCR 法具有快速、简便、经济等优点,是一种非常快捷的扩增 cDNA 3′末端序列的方法。

关键词:一步 PCR;辽宁碱蓬;甜菜碱醛脱氢酶;3′cDNA 末端

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Rapid Amplification of 3′cDNA End of *Suaeda liaotungensis* Betaine Aldehyde Dehydrogenase Using One-step PCR

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Abstract: Based on part of a known cDNA sequence of *Suaeda liaotungensis* betaine aldehyde dehydrogenase, we successfully cloned the 3′cDNA end of S. *lianotungensis* betaine aldehyde dehydrogenase using one-step PCR with a gene-specific primer and universal primer. Compared with the typical 3′ RACE, one-step PCR is rapid, simple and inexpensive. It is very rapid to amplify an unknown cDNA 3′end using this method.

Key words: one-step PCR; *Suaeda lianotungenesis*; betaine aldehyde dehydrogenase; 3′cDNA end.

In 1985, Mullis and his colleagues of Human Genetic Laboratory of PE－cetus Company in America invented Polymerase Chain Reaction (PCR) which can unlimitedly amplify nucleotide fragment in vitro. More than a decade later, PCR technique had been well improved and applied to the life science researches world wide. Many PCR techniques, such as Reverse Transcript PCR, Inverse PCR, Anchored PCR, Nested PCR, and DDRT－PCR, were successfully used in amplifying cDNAs and genomic genes, analyzing RNA expression, genetic diagnoses, mutant assay, genetic engineering *et al*[1]. In today's "Gene War", discovering and cloning of new genes have become the research hotspot, and in those researches, the PCR was the major tool in amplifying unknown sequences. Rapid Amplification of cDNA Ends (RACE) technique has the most advantages among all mentioned techniques. It can rapidly produce 5′and 3′ends of targeted fragments. We obtained a betaine aldehyde dehydrogenase (BADH) 3′end cDNA of *Suaeda liaotungensis* using a improved 3′RACE with only one step PCR.

1 Materials and Methods

1.1 Materials

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Plants of *S. liaotungensis* were collected from seaside of Dalian. Primers were designed by our lab and were synthesized by TaKaRa Biotechnology Dalian Co., Ltd. Reagents were purchased from GIBICOBRL and TaKaRa.

1.2 Methods

1.2.1 Extraction of total RNA

Total RNA was extracted from leaves of *S. liaotungensis using* TRIZOL kit(GIBICOBRL).

1.2.2 Synthesis of the first strand of cDNA

An universal RT primer(oligo dT—adapter Primer) was used to synthesize the first strand of cDNA. The reagents and their amount used in RT reaction were 1μl total RNA (500ng/μl), 2μl 10×RNA PCR buffer, 4μl $MgCl_2$ (25mmol/μl), 0.5μl Rnase Inhibiter (40U/μl), 1μl AMV Reverse Transcriptase XL(5U/μl)(TAKARA), 1μl Oligo dT—adaptor Primer (2.5pmol/μl), 2μl dNTP Mixtrue (10 mmol/μl each) and 8.5μl Rnase Free dH_2O. The reaction conditions were as follows: 42℃ for 30min, 99℃ for 5min, and 5℃ for 5min. This first strand cDNA was used as PCR template.

1.2.3 PCR reaction

According to the known partial sequence of *S. liaotungensis* BADH, a gene—specific primer was designed near its 3′— end and named F1: 5′— AGTACCGAAGAGGAAGCCCTT—3′. The reagents and their amount used in the PCR reaction were 1μl first strand cDNA, 5μl 10×LA PCR Buffer, 16μl dNTP Mixtrue(2.5 mmol/μl each), 0.5μl TaKaRa LA *Taq*(5U/μl), 1μl F1(20pmol/μl), 1μl M13 primer M4 (20pmol/μl), and 69μl ddH_2O. The sample was heated at 94℃ for 1 min and cycled for 30 times at 98℃ 10 sec, 50℃ 30 sec; 72℃ 1min, followed by a 72℃ 7min step.

1.2.4 Recovery and sequencing of PCR products

PCR products were recovered by using NaI recovery kit (TaKaRa), and then sequenced by TaKaRa Biotechnology Dalian Co., Ltd.

2 Results

2.1 PCR results

The 1% agarose gel electrophoresis of the PCR products showed a 600bp band(Fig. 1).

2.2 Sequencing and sequence analyses

A 552bp nucleotide sequences were detected in the PCR products. It was betaine aldehyde dehydrogenase 3′end cDNA of *S. liaotungensis*. This sequence included C terminal sequence which encodes 73 amino acids, the translation stop code TAA, and 3′UTR 329bp which included poly(A) signal and poly(A) tail(Fig. 2).

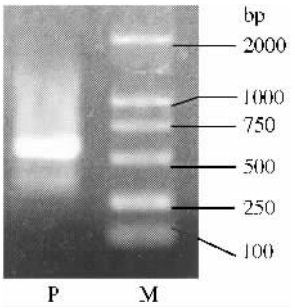

Fig. 1 Electrophoresis of PCR

(M: DL 2000 DNA Marker; P: Product of PCR)

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AAAGACCTTGAAAGGTGTGAGAGGGTAACAAAGGCTCTAGAAGTTGGGGCTGTCTGG
 K  D  L  E  R  C  E  R  V  T  K  A  L  E  V  G  A  V  W
GTGAATTGCTCACAGCCATGCTTTTGCCATGCTCCATGGGGAGGCGTCAAGCGTAGC
 V  N  C  S  Q  P  C  F  C  H  A  P  W  G  G  V  K  R  S
GGTTTTGGACGTGAGCTTGGAGAATGGGGTATTGAAAATTACTTGAACATTAAGCAA
 G  F  G  R  E  L  G  E  W  G  I  E  N  Y  L  N  I  K  Q
GTGACTAGCGATATTTCCGATGAACCATGGGGGTGGTACAAGTCTCCTTAAaggcaa
 V  T  S  D  I  S  D  E  P  W  G  W  Y  K  S  P
aagaggatatttgcaagataatgctgttatcaagtgaactgtgacacaagagtgacg
acccatgtaatgttgtataacgatctagctcacagtttgtctatttgattaaataag
ggtcgtgcgatgctggagttccataggcattgattgattttgctatttgtgttattt
tggaccattgagaaaatttttggaccagggataagatgcttgcatataacattaagc
ctgttatatttgcaagtttaaattatatttgggtgtgttatgtaactaatgtttcat
taataaaattctccttcgtctcgaaaaaaaaaaaaaaaa
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Fig. 2 Nucleotide and deduced amino acid seduences of BADH 3′cDNA End

3 Discussion

Obtaining full—length cDNA is the essential foundation of the research on gene structures, gene expressions and gene functions. Various protocols have been developed to isolate unknown cDNAs, but most of times we only get partial sequences without the whole Open Reading Frame[2]. The traditional method to isolate a full—length cDNA is to prepare a cDNA library. Based on this library, specific gene probes are synthesized. Then probes are used to screen and analyze the positive—reaction clone. This method is laborious, time consuming and expensive, and the full—length cDNA could not always be obtained. With the development of the Internet, the Electronic Clone Technique has been invented. It is rapid and convenient, but it can only be used in a few species whose genomic sequences are known. So it has narrow applicability. Compared with the above two techniques, RACE developed

at the end of 1980s is rapid, simple, efficient, and inexpensive. Using this technique, a full－length cDNA can be obtained in 2～3 days. Two universal primers and one gene－specific primer were designed in typical 3′ RACE. One universal primer is Oligo dT－adaptor Primer, the other is adaptor Primer. The gene－specific primer and OligodT－adaptor Primer were used in first PCR reaction, and then the products were used as template of second PCR with the gene－specific primer and adaptor primer[3]. Sometimes two gene－specific primer were designed and used in first and second PCR, that is, nested PCR was used to reduce non－specific amplification[4]. In this method polycyclic amplifications increased the miscorporation rate, moreover it is time consuming. We modified the above typical method. The universal primer was used in RT reaction to obtain first strand cDNA. Then the first strand cDNA was used as template in following PCR with a gene－specific primer and universal primer. In this method it needed only one step PCR, so it was rapid and it has better specificity. We obtained a new *S. lianotungenesis* betaine aldehyde dehydrogenase cDNA 3′ end using this metod. One－step PCR has been known as an efficient, rapid and inexpensive method of cloning unknown 3′end cDNA.

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