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RESEARCH ARTICLE

A SIMPLE STRATEGY FOR PCR PRODUCTS IDENTIFICATION

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ABSTRACT

The false-positive PCR DNA fragments are often obtained due to many factors. Whether the DNA fragments were the target need to be identified by some methods, such as electrophoresis, sequencing eta. Here, we describe a simple PCR amplified products indentified method by endonuclease enzyme digestion. The DNA fragments were amplified by PCR method, then digested by endonuclease enzymes that determined by internet Software. If the digested products were consistent with the analysis of Software, they were sequenced further. We used the surviving promoter as instance to illustrate this method.

INTRODUCTION

The polymerase chain reaction (PCR) is a scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. Developed in 1983 by Kary Mullis, PCR is now a common and often indispensable technique used in medical and biological research labs for a variety of applications (Saiki *et al.,* 1985; Saiki *et al.,* 1988; Bartlett and Stirling 2003). These include DNA cloning for sequencing, DNA-based phylogeny, or functional analysis of genes; the diagnosis of hereditary diseases; the identification of genetic fingerprints (used in forensic sciences and paternity testing); and the detection and diagnosis of infectious diseases. Although PCR technology has been quite common and mature now, the target PCR products could not often obtain because there are many various factors that influence the PCR reaction conditions, such as Mg^{2+}, dNTPs, primer, templates, cycle parameters, etc, especially for some genomic DNA complex templates. Design of primer is one of the key factors in PCR reaction, good primer design is essential for successful reactions. Some preferred values must be considered during the primer design, including length, melting temperature, annealing temperature, GC content, GC clamp, secondary structures, and 3' end stability, etc (Brown *et al.,* 2010; Li *et al.,* 2011).Higher eukaryotic organism genome DNA structure is more complex, and there are lots of repeat sequences, so sometimes even primer accords with the design principle, but the PCR could not successful .

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PCR products are often identified initially by agarose gel electrophoresis, the size of them could be compared to the expected, then sequencing. If the amplified DNA fragments are only judged by the size on the electrophoresis, the result is not reliable (Wang *et al.,* 2004). Most labs have no the sequencing instrument, especially to the labs in the developing countries, the DNA sequencing must be completed by the professional gene company. In the present study, we describe a method of indentifying PCR products through enzyme digestion.

MATERIAL AND METHODS

Cell culture and genomic DNA extraction

Hela cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS), 5% CO_2 at 37ºC. When they reached 80 – 90% confluence, cells were washed with ice-cold PBS and homogenized immediately. Hela cell genomic DNA was extracted according to the previous described methods(Wang *et al.,* 2011).

Primer design and PCR amplification

The survivin primers were designed according to the surviving promoter sequence (GenBank no: U75285.1) (Zu *et al.,* 2011), and the sequences were as follows: P1:5′-GCCATTAA TCTGGCCATAGAACCAGAGAAGTGA-3′;P2:5′-ATACG CTAGCCGCACGCCCTCTTAGGCGGTCCACC-3′. The amplifications were performed using the standard reaction system. The PCR reaction was performed under the following

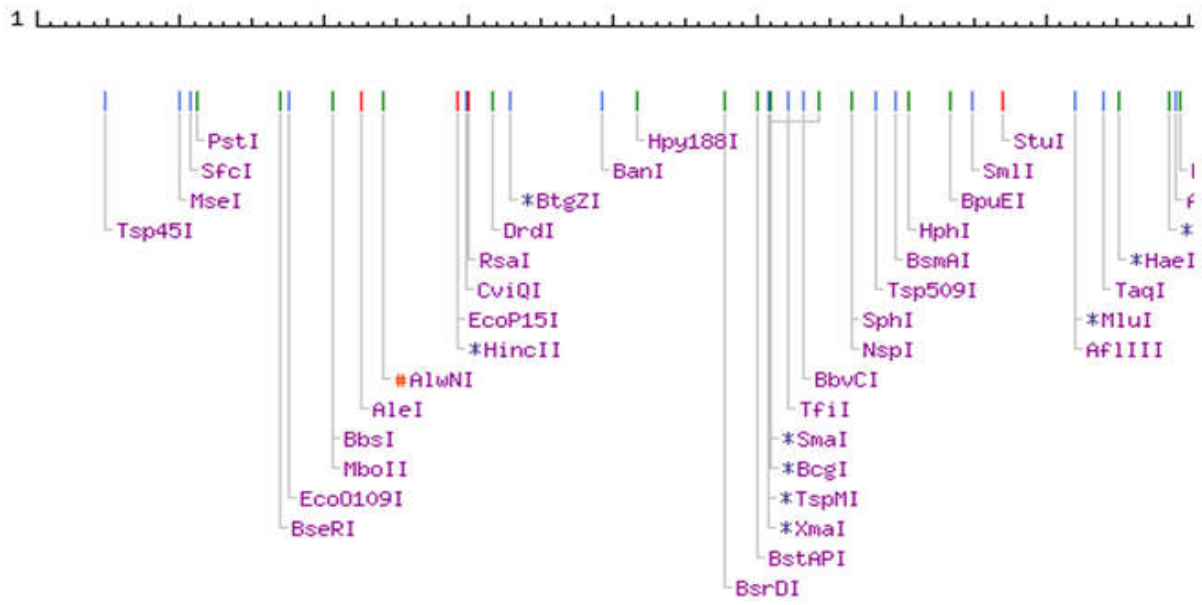

Fig. 1. The analysis result of the sequence of the survivin promoter enzymes sites

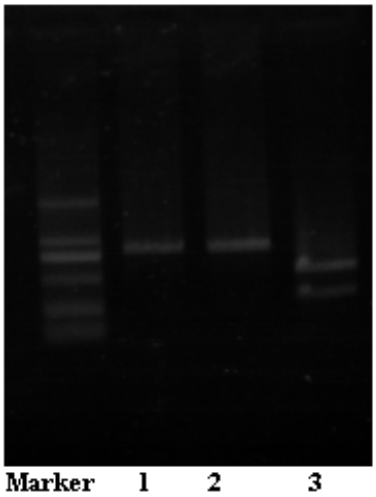

Fig. 2. The PCR application of the survivin promoter and the result of the digestion with restriction enzymes. Lane 1: PCR products of the survivin promoter; 2: Digested by HindIII; 3: Digested by SmaI

conditions: 95℃ for 5 min followed by 30 cycles of 94℃ for 40 s, 50℃ for 30 s and 72℃ for 40 s, and finally 72℃ for 5min.

Analysis of enzyme sites and digestion reaction

The restriction enzyme sites of surviving promote sequence were performed by the http://tools.neb.com/ NEBcutter2/ index.php), and the restriction enzyme digestion protocol was carried out according to the standard conditions (Joseph and David, 2001).

Recovery and sequencing

A 5-μL aliquot of each PCR reaction mixture was screened by 2% agarose gel electrophoresis, and the length was estimated by comparing with a known size DNA marker (Sangon), followed by purification using the recovery kit (TakaRa, Dalian, China)and sequencing by the Sangon Shanghai Company. BLAST was done at the NCBI server (http://www.ncbi.nlm.nih.gov/ blast/ Blast.cgi)

RESULTS

Enzyme sites analysis of surviving promoter

We analyzed the enzyme sites of surviving promoter sequence through the NEB web software (http://tools.neb.com /NEBcutter2/index.php). The results showed that there are 38 enzyme sites, some of them are unique site, such as BanI, Sma I, Mlu I ert. We selected Sma I enzyme site in our experiment. If the DNA fragments was digested by the *Sma* I enzyme, they would produce 511 bp long and 400bp two fragments (Fig. 1). However, the DNA fragment will generate one single DNA fragment if digested by *Hin* dIII enzyme site because the enzyme site does not exist.

PCR amplification and enzyme digestion

The surviving promoter was amplified by the method described above. The size of PCR-amplified product was consistent with the expected result (Figure 2).We carried out the PCR products recovery and enzyme digestion, the results showed that the only one DNA fragment appeared when digested by HindIII, but two DNA fragments of about 511bp and 400bp were shown when cut with Smal enzyme, suggesting that the cloned DNA fragments was the target DNA fragment.

Sequencing and analysis result

The purified DNA fragments were sequenced and analyzed further, the results demonstrated that the sequence was the same as the reported sequence.

DISCUSSION

As a kind of DNA amplification method of in vitro, PCR has the advantages of sensitive, peculiar, simple, convenient, and rapid etc(Wang *et al.,* 2004), which has been widely in used biomedical studies. Primer is one of the important components of PCR system that influence PCR results. Good primer design is essential for successful reactions. Good primer in the template binding sites should be the only, only so could assure the amplified product specificity. According to the general rule of primer design, if the primer contains 15 nucleotides, the primer could only complement the genomic DNA of $3\times l0^9$ bp mammalian genomes completely at one site. However, due to the distribution of nucleotide sequence of eukaryote genes is not random. Codon preference and a lot of repeat sequences and gene families exist, which makes the PCR often produce non-specific product. Even the primer length is more than 20 nucleotides; there are only 85% of sequences that can be accurately matching in mammalian genomes (Joseph and David, 2001). For this reason, PCR can sometimes produce the non-specific amplification product, sometimes the band and expected size is consistent through identification by the electrophoresis, but the sequence results are not the target DNA fragment (Saiki *et al.,*1985). At present the most reliable DNA fragments is confirmed according to sequencing results, but most laboratory could not carry out this experiment. And due to some factors, not every sample can be sequenced timely and correctly.

To take this described method in the present paper, we can identify the amplified PCR product correctly preliminary, which need not expensive laboratory requirement, and most laboratories can perform this experiment.

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