Simple 5’ Extension Method Allowing Transcript Discrimination

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To analyze plant genes expressed during an incompatible interaction between leaves of Medicago sativa and Pseudomonas syringae pv. pisi (2), we constructed a Agt10 cDNA library using cDNAs ligated to an EcoRI adaptor (Amersham, Les Ulis, France). Results indicated that many of the genes analyzed belonged to multigene families and that some of the cDNA clones isolated from this library lacked from 50- to 400-bp-long fragments of the corresponding mature transcripts, as was the case for cDNAs coding for chalcone synthase (2), chalcone reductase (6) or peroxidases (3).

A number of protocols [such as random amplification of cDNA ends (RACE) (1,4,5), ligation-anchored polymerase chain reaction (PCR) (8) and inverse PCR (7,9)] have been devised to solve these problems, which include 5’ incomplete cDNAs and transcript discrimination; however, we were not successful in using some of those protocols. Having reasoned that the existence of an EcoRI adaptor at both ends of the cDNA molecules might help to solve these difficulties, we developed (Figure 1) a PCR approach using one primer specific for the adaptor (upper primer, 5’→3’) and another specific for a sequence within the cDNA of interest (lower primer, 3’→5’, complementary to the + strand). Compatible between primers and annealing temperatures were checked using the Oligo™ Analysis Software (National Bio-Sciences, Plymouth, MN, USA).

Experimental conditions. Treatment of plants with bacterial suspensions, extraction of mRNA and preparation of the Agt10 library were as described previously (2). PCR amplification was carried out in a Crocodile II Thermocycler (Appliènne, Illkirch, France). Typically, 1 ng of total cDNA ligated to an EcoRI adaptor was combined with 70 pmol of each oligonucleotide primer (O.adap, O.sp) in a 50-µL reaction mixture (10 mM Tris-HCl, pH 9.0; 50 mM KCl; 1.5 mM MgCl₂; each dNTP at 200 µM; 0.1% Triton® X-100; 0.2 mg/mL bovine serum albumin [BSA]), followed by the addition of 0.2 unit of Taq DNA polymerase (Appliènne) and 50 µL of mineral oil. Reaction conditions (standard amplifications) were 5 min at 95°C and 25 cycles of 95°C for 1 min, 53°C for 2 min and 72°C for 2 min.

The technique was first applied to amplify the 5’ fragment of a cDNA encoding a class of PR (pathogenesis related) proteins by using the EcoRI adaptor-ligated cDNA population made for preparing the cDNA library and an oligonucleotide specific for an incomplete cDNA clone, designated as PR7 and corresponding to a transcript of approximately 0.8 kb (2). As shown in Figure 2, lane A, the amplified products corresponded to the expected size, i.e., 270 bp. Upon further amplification (ca. 25 cycles), the specific product yield was reduced and a smear appeared (data not shown). Cloning and sequencing the various subclones obtained from this DNA fragment revealed (Figure 3A) that it contained at least two sequences (designated as C16 and C18) encoding a PR protein. These sequences, encompassing the putative ATG codon and differing by 3 nucleotides, led to a single change (N/T) in the amino acid composition. The C16 clone is most likely equivalent to the PR7 cDNA and the C18 may be an allelic variant. A smear, ranging from approximately 300 to 1500 bp, appeared (Figure 2, lane B) when the oligonucleotide specific for the cDNA of interest was omitted. This smear corresponded to the amplification of the cDNA population.

To illustrate the general applicability of this technique, we used an oligonucleotide deduced from a highly conserved region of plant peroxidases. As shown in Figure 3B (for sake of clarity the deduced amino acids are presented only), sequencing of a number of clones (E6, E8, F3) from a 350–400-bp amplified DNA fragment resulted in three different sequences. They differed both in length and in amino acid composition from the reference clone, PRX1 (3). These sequencing data suggest that the transcripts corresponded to different genes (PRX1 vs. E6, E8 or F3) or allelic variants (E6 vs. E8). However, by using an oligonucleotide

Figure 1. Scheme of PCR amplification for 5’ extension. Oligonucleotide primers: the EcoRI adaptor sequence (O.adap) and a known sequence within the cDNA of interest (O.sp). The Amersham EcoRI adaptor and the deduced oligonucleotide are also shown.

Figure 2. Agarose (1.2%) gel electrophoresis. (M) molecular weight markers; the sizes (in bp) are indicated. (A) PCR amplification obtained using the O.adap and O.sp. (B) Products obtained by using the O.adap only. The gel was stained with ethidium bromide (EtBr) to visualize DNA fragments.
Benchmarks

specific for the PRX1 clone, we were able to extend this clone to the 5′ end of the transcript (data not shown).

In conclusion, our approach to extend incomplete clones from a cDNA library is particularly simple and suitable to discriminate transcripts, obtained from various genes or allelic variants encoding a known protein and expressed under a given physiological condition, from a population of cDNAs ligated to the EcoRI adaptor, thus avoiding the preparation of a library. Moreover, this method seems to be simpler and less time-consuming than the other methods reported so far and may be applied to any adaptor-ligated cDNA population, provided the adaptor is suitable for designing a useful primer.

REFERENCES


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