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analyzing the gene of interest has several advantages over subcloning analysis. This method does not require any information about physical maps of the insert DNA. Positions of transposon insertion are presented as a distance from one end of the genomic DNA. Even if the restriction map is known, this technique should be preferable because of its simplicity. Using this protocol, it takes only one week to identify the region essential for the complementation. Furthermore, restriction digestion or ligation reaction is not necessary to determine the partial DNA sequence of the gene. Therefore, with this protocol, one can reduce the time required for DNA cloning and move on to the functional analysis of the gene. Finally, this protocol should prove useful for cloning in other organisms in which cloning by complementation is popular.

REFERENCES

1. Barnes, W.M. 1994. PCR amplification of up to 35-kb DNA with high fidelity and high yield from lambda bacteriophage templates. *Proc. Natl. Acad. Sci. USA* 91:2216-2220.
2. Carlson, M. and D. Botstein. 1982. Two differentially regulated mRNAs with different 5' ends encode secreted and intracellular forms of yeast invertase. *Cell* 28:145-154.
3. Cheng, S., C. Fockler, W.M. Barnes and R. Higuchi. 1994. Effective amplification of long targets from cloned inserts and human genomic DNA. *Proc. Natl. Acad. Sci. USA* 91:5695-5699.
4. Feldmann, H., M. Aigle, G. Aljinovic, B. Andre, M.C. Baclet, C. Barthe, A. Baur, A.M. Becam et al. 1994. Complete DNA sequence of yeast chromosome II. *EMBO J.* 13:5795-5809.
5. Ohya, Y. and D. Botstein. 1994. Diverse essential functions revealed by complementing yeast calmodulin mutants. *Science* 263:963-966.
6. Ohya, Y., S. Miyamoto, Y. Ohsumi and Y. Anraku. 1986. Calcium-sensitive *cls4* mutant of *Saccharomyces cerevisiae* with a defect in bud formation. *J. Bacteriol.* 165:28-33.
7. Sambrook, J., E.F. Fritsch and T. Maniatis. 1989. *Molecular Cloning: A Laboratory Manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
8. Sasakawa, C. and M. Yoshikawa. 1987. A series of Tn5 variants with various drug-resistance markers and suicide vector for transposon mutagenesis. *Gene* 56:283-288.
9. Smith, V., D. Botstein and P.O. Brown. 1995. Genetic footprinting: a genomic strategy for determining a gene's function given its sequence. *Proc. Natl. Acad. Sci. USA* 92:6479-6483.
10. Yoshihisa, T. and Y. Anraku. 1989. Nucleotide sequence of *AMS1*, the structure gene of vacuolar alpha-mannosidase of *Saccharomyces cerevisiae*. *Biochem. Biophys. Res. Commun.* 163:908-915.

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Identification of Introns by Reverse-Transcription PCR

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Analysis of previously unidentified genes is often begun by screening an expression library with a specific antibody or screening a cDNA library with a heterologous probe. The advantage of using cDNA libraries is that the identified clone will contain only coding sequences. However, there are three instances where the use of a cDNA library is not practical. First, if the gene under investigation is a regulatory gene and the level of mRNA is low, the gene might not be represented in the cDNA library. Second, if the gene is long and the first-strand cDNA synthesis did not go to completion, the 5' region of the gene might not be represented in the library. Third, if the gene has a developmentally regulated pattern of expression or a limited tissue specificity, it is possible that the cDNA library does not represent the time or tissue specificity of peak expression of the gene, thus minimizing the chance of finding the clone in an available library.

Cloning from a genomic library or directly from a genomic DNA preparation can circumvent all these problems.

However, there is always the possibility that the genomic clone will contain intervening sequences. These sequences are currently identified by comparing the sequence of genomic and cDNA clones. However, as stated above, cDNA clones are not always available. In the following note, we suggest a rapid and accurate method to identify introns within a cloned genomic sequence in the absence of a cDNA clone.

In our efforts to identify the *Dictyostelium* homolog of the primate UV-damaged DNA-binding (UV-DDB) protein (1), we screened a cDNA library and identified a series of positive partial clones representing a 1.6-kb 3' end of the gene. An exhaustive screen of the library did not reveal any longer clones. We therefore constructed a detailed restriction map of the region

Figure 1. A) Schematic presentation of the *repE* gene. Oligonucleotides that were used for PCR are depicted by arrows. Oligo #6 was used for reverse-transcription (RT)-PCR. The position of the 92-bp intron is shown. B) PCR amplification of genomic DNA and cDNA. PCR was performed using a Coy Laboratory Products thermal cycler (Grass Lake, MI, USA) for 30 cycles under the following conditions: 1-min denaturation at 94°C, 2-min annealing at 40°C and 3-min elongation at 72°C. The last elongation was extended to 10 min. Each reaction contained 2 μ L of 20 μ M primers, 10 μ L of 2.5 mM dNTP and 1 μ L *Taq* DNA polymerase (AmpliTaq®; Perkin-Elmer, Norwalk, CT, USA) in buffer containing 20 mM Tris-HCl, pH 8.4, 50 mM NaCl and 1.5 mM $MgCl_2$. For the amplifications of genomic sequences, we used 2–3 μ g of total *Dictyostelium* genomic DNA per reaction. For the amplification of the cDNA, we used 2.2 μ L of the RT reaction, following the instruction in the kit. The following sets of primers were used: set 1, primer 44→primer 55; set 2, primer 44→primer 57; set 3, primer 14→primer 59 and set 4, primer 10→primer 29. In each set, left lane represents cDNA (c) and right lane represents genomic DNA (g). Molecular weight markers are indicated.

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around the gene and cloned the entire gene from genomic DNA. Sequencing the genomic clone showed that there was an additional 1.8 kb of a 5' sequence to our original cDNA sequence. In addition, comparison of the genomic sequence to the cDNA sequence revealed the presence of a 92-bp intron in the 3' half of the gene. Because of the absence of an available cDNA sequence for the 5' half of the gene, we could not directly determine the presence of additional introns in this part of the gene. We therefore used the following method for screening the remaining genomic sequence for introns.

Total RNA samples from a developmental time point of peak expression of the UV-DDB homolog were treated with DNase I to remove any contaminating DNA in the samples. The mRNA was then reverse-transcribed to generate the first strand of cDNA. In our experiments we used a first strand

cDNA synthesis kit of Life Technologies (Gaithersburg, MD, USA) and oligonucleotide #6 as a primer (Figure 1A). The resulting cDNA as well as genomic DNA were then amplified using PCR and four primer sets that span the entire gene as shown in Figure 1A. The PCR product of the cDNA and the genomic DNA were compared on 1.2% agarose gels as is shown in Figure 1B.

By using this method, we have confirmed the presence of the one intron at 1.2 kb from the 3' end of the gene (set 4) and verified that the rest of the genomic clone contained no introns (primer sets 1, 2 and 3).

This method is particularly applicable in organisms where introns are neither very abundant nor very long. If an intron is encountered, it is easy to generate a large amount of PCR product for direct sequencing. Thus the exact splice sites and the actual coding sequences can be identified.

REFERENCE

1. Abramic, M., A.S. Levine and M. Protic. 1991. Purification of an ultraviolet-inducible, damage specific DNA-binding protein from primate cells. *J. Biol. Chem.* 266:22493-22500.

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