

A simple method for generating full length cDNA from low abundance partial genomic clones

ABSTRACT

We have devised a simple, rapid and easy method for generating cDNA clone from genomic sequences. The full length HOXD13 clone (1.1 kb) generated with this technique was confirmed by sequence analysis. This simple approach can be utilized to generate full-length cDNA clones from available partial genomic sequences.

INTRODUCTION

Background The first approach we used for the isolation of full length cDNA from two known genomic clones of Hox genes was based on fusion PCR. It involved selection of primers at the 5' and 3' ends of the two exons with a complimentary set of primers in the middle of the clone. This approach was time consuming and labor intensive, especially with the number of different combinations of primer pair sets and series of PCR amplifications. We were successful in generating the 3' half of the clone with this procedure, but the high GC content in the 5' end of the clone proved to be a challenge with this strategy. To circumvent this problem, we used 5'-RACE (Rapid amplification of cDNA ends) procedure to generate 5'-end of the clone. This method involved use of many more specific primers, nested gene-specific primers, followed by PCR amplifications of homopolymer-containing anchor/adaptor priming steps. While considerably more efficient than fusion PCR, this technique was still unable to generate the specific high GC rich 5' end of our gene of interest i.e., the homeobox D13 cDNA. Here we describe a novel and efficient PCR-based method for the generation of full length cDNA of low abundance transcripts from two partial genomic clones. Our approach took advantage of three aspects: 1) selection of the two exons from two individual clones without intervening intron sequences, 2) generation of a contiguous sequence representing the potential full length cDNA sequence having both 5' and 3' untranslated regions (UTR) and 3) utilizing the website program(primer3_www.cgi.v0.2) to design specific primers within 5' and 3' UTRs to generate a full length cDNA clone of about 2 kb (fig. 1). A major advantage of this technique is the species sub-group specificity provided by the 5' end primer used in the amplification. However, in order to increase total cDNA random hexamers can be used. The specificity permits the amplification of a particular clone in the presence of other closely related gene family members. We have applied this technique to prepare a full length homeobox D13 cDNA clone from two genomic clones isolated by Goodman and coworkers.

CONCLUSION

Conclusions In summary, we have developed a simple, rapid and easy to perform PCR-based procedure to generate full length cDNA clone from two partial genomic clones. Although we have demonstrated the use of the technique by preparing a full length HoxD13 cDNA clone, the procedure could be easily adapted to other complex subsets of low abundance genes. A major advantage of this method over existing ones is its simplicity, specificity and precision, which eliminates the need for several intermediate enzymes and steps to generate a full length clone.