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

ABSTRACT

We have developed a simple, fast and easy way of generating cDNA clone from genomic sequences. The full-length HOXD13 recombination (1.1 kb) produced using this method was confirmed by sequence analysis. This simple approach can be used to generate full–length copies of available partial genomic sequencing.

INTRODUCTION

The high level of variation among human genomes is an important source of evolutionary constraint on the evolution of human traits. The ability of humans to maintain their genetic diversity in different environments is an important aspect of human evolution. The ability of the human genome to maintain high levels of genetic diversity when compared to other mammals, including humans, is an important source of evolutionary constraint on the evolution of many mammalian traits. The cDNA sequence of a human genome can be used to generate a high-quality full-length cDNA sequence from a low abundance partial genomic clone of a human. This is an example of how natural selection can influence the evolution of human traits. In this paper we describe an approach that allows the generation of highly complete cDNA sequences from low abundance partial genomic clones.

Methods:

A sample of the human genome Our strategy for isolating full length genomic cDNA from two known clones of Hox genes was initially achieved through fusion PCR, but it was ultimately unsuccessful due to the high GC content and time-consuming process. We now use 5'-RACE (Rapid amplification of cleaver/patchgun hybridization) to generate 5’end of the chromosomes rather than 3' genome sequences. This method was much more efficient because it involved multiple sets of primers, including both orthologisation and also the same number of highly ang We utilized three factors: 1) the extraction of two exons from consecutive clones without interfering intron sequences, 2) the creation of a contiguous sequence that represents the possible full-length cDNA sequence with untranslated regions 5' and 3' (UTR), and 3) the use of the program (primer3_www.cgiv0.2) to design primers within 5’ and 2' UTRs; all of which were designed to create essentially RNA-free PCR products. One of the main advantages of this technique lies in the sub-species specificity of that 5' end primer used for amplification; however, it can be used to generate more total (say 396 genes or more) of total cDNA random Hexamers, which means they can also be amplified in presence of other closely related gene family members. Thus we have applied this method to prepare clone 1 (full length homeobox D13) on two isolated genomics chromosomes isolated by Goodman and coworker.

CONCLUSION

Remarkable conclusions Essentially we have devised a simple, rapid and easy to follow PCR-based procedure for cloning full length cDNA from two partial genomic oligosaccherogens (in that case we firstly prepared the fulllength HoxD13 CCNA; in contrast, we also demonstrated how this technique could be easily applied to other complex subsets of low

abundance genes, since it does not require many steps and intermediary enzymes to complete whichever one is produced).