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A simple method for direct cloning and sequencing cDNA by the use of a single specific oligonucleotide and oligo(dT) in a polymerase chain reaction (PCR)

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CDNA cloning by PCR with the use of two mixed oligonucleotides corresponding to the same mRNA has been described recently (1). If only one short peptide sequence is known or when the 3'noncoding segment of a mRNA is the goal of the cloning effort, different strategies have to be applied. In genomic cloning, 3'most exons may be difficult to obtain directly from lambda libraries when they are separated from the next upstream exon by large introns. In the case of the rat parvalbumin gene, a 9 Kbp intron precedes the 3'most exon containing the TAA translation termination codon which is located 26 bp downstream from the splice site (2). In human parvalbumin genomic clones isolated by the use of rat full length parvalbumin cDNA (3) as a probe, the 3'most exon could not be found. This indicated the presence of a large intron also in the 3'region of the human parvalbumin.

The method reported here was designed to clone the 3' region of the human parvalbumin gene but is suitable for cloning any cDNA especially when only one short amino acid (eg. N-terminal of a protein) or nucleic acid sequence is known, when the abundance of the transcript of interest is low or when the RNA source is limited. In addition, this method allows the amplification of sequences from cDNA libraries without the need for plating bacteria or phages.

1. First strand cDNA synthesis: 0.2 µg of poly(A+) RNA from human cerebellum with 5 µg E.coli carrier tRNA were reverse transcribed as reported (3) in the presence of 2 µM oligo(dT-20) with an Xba 1 site at the 5'end.
2. PCR: After RNA hydrolysis (1), 1/10 of the sythesized cDNA containing approximately 60 to 600 fg of parvalbumin specific cDNA was used in a PCR reaction according to Saiki et al. (4) with 1 U Taq 1 polymerase (Anglian Biotechnology, GB) and 2 µM each of the same oligonucleotide as described above in 1. and a 20 nt synthetic oligonucleodide corresponding to human parvalbumin exon 4, with a Sal 1 restriction site at the 5'end. PCR was performed for 30 cycles (annealing 2 min at 40 °C , elongation 2 min at 72 °C, and denaturation 1 min at 94 °C) with a PCR PROCESSOR (BioMed, 8729 Theres,FRG). Approximately 600 ng of specific reaction product were accumulated during the PCR. This corresponds to an amplification of 10^6 to 10^7.
3. Cloning and sequencing: After a final 10 min. elongation, the reaction product was phenol extracted and digested with Sal 1 and Xba 1. DNA of 280 to 300 bp with varying length of poly(dAdT) 3'tails was excised from an agarose gel and electroeluted using a Biotrap^R apparatus (Schleicher & Schuell, FRG) and either ligated into the Sal 1 and Xba 1 sites of M13mp18 and pGEM3 vectors or directly sequenced. Standard dideoxy sequencing protocols were used either on double stranded DNA with the upstream PCR primer or on M13 clones with the M13 universal primer. The sequence obtained was identified as human parvalbumin cDNA by homology to the rat counterpart.

Figure: CDNA derived from poly(A+) of human cerebellum (67 years old male, 8 h post mortem) synthesized in the presence of ^{32}P dCTP (lane 1) and human parvalbumin cDNA, 3'region, after 25 (lane 2a) and 30 cycles (lane 2b) of amplification. CDNA aliquots were separatred on a 1.5% agarose gel and blotted onto a nylon membrane followed by autoradiography. Molecular weight standards (pBR322/Hpa II) are given in bp, O = origin of loading.

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