RAPID AMPLIFICATION OF cDNA ENDS (RACE)

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ABSTRACT

Rapid amplification of cDNA ends is a polymerase chain reaction-based technique which was developed to facilitate the cloning of full-length cDNA 5' and 3' ends after a partial cDNA sequence has been obtained by other methods. While RACE can yield complete sequences of cDNA ends in only a few days, the RACE procedure frequently results in the exclusive amplification of truncated cDNA ends. Many investigators have suggested modifications to the RACE protocols to improve the effect of the technique.

Keywords: RACE, PCR

1. INTRODUCTION

Since the polymerase chain reaction (PCR) was developed and the basic reaction conditions were optimized, the technique has been applied to many aspects of molecular biology research. One of the most useful applications of PCR is the amplification and subsequent cloning of low-abundance DNA sequences from complex mixtures, even using as little as a single cell as the source of genetic material. Previously, such cloning projects involved the arduous task of creating a phage or plasmid library and screening millions of plaques or colonies to obtain overlapping cDNA representing the full-length sequence. This procedure often requires many months of work to isolate the desired sequence in entirety. The advent of PCR has made it possible to reduce this time to weeks or days.

This review focuses on the application of PCR to the cloning of unknown flanking cDNA sequences when a short fragment of cDNA is already known. There were several independent descriptions from researchers who named their techniques as rapid amplification of cDNA ends (RACE) [1], anchored PCR [2], and one-side PCR [3]. There have been several modifications of he original methods, including ligation anchored PCR (LA-PCR) [4],

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single-strand ligation to ss cDNA ends (SLIC) [5], RNA ligase-mediated RACE (RLM-RACE) [6], reverse ligation-mediated PCR(RL-PCR) [7] and circular or concatemeric first-strand cDNA–mediated RACE (cRACE) [8]. By slightly different applications of the protocols, unknown sequences both 5' and 3' of the known sequence can be reverse transcribed from RNA, amplified by PCR, and cloned.

2. PRINCIPLE OF RACE

Frohman [1] and Loh [2] initially described the rapid amplification of cDNA ends(RACE) procedure for cloning unknown cDNA ends respectively. To clone an unknown 5'-end sequence, cDNA is synthesized from an RNA sample using either oligo(dT) or an oligonucleotide primer complementary to a known sequence in the target mRNA. The 3'-end of the first strand cDNA is modified by the addition of a homopolymer tail using terminal deoxynucleotidyltransferase(TdT).

The cDNA second strand is then synthesized using a primer consisting of a cloning sites adaptor having a 3'-homopolymer tail which is complementary to the appended cDNA 3'-homopolymer. These double strand cDNA are amplified by PCR, employing the cloning adaptor as the 5'-end primer and an oligonucleotide complementary to a known sequence internal to the cDNA primer as the 3'-end PCR primer. An aliquot of the products of the PCR is then separated by electrophoresis through an agarose gel, and specific products are identified, cloned and sequenced by established methods.

3. WEAKENESS OF THE ORIGINAL RACE

Although the original RACE has proven extremely valuable to many investigators, many labs have found it is difficult to successfully apply these protocols. Generally speaking, there are two reasons for this high failure rate. The first is that there are three sequential enzymatic steps (reverse transcription, TdT tailing and PCR), each of which is subject to partial or complete failure. The second is that even when the enzymatic syntheses proceed smoothly, there is often a high background of nonspecific or truncated product generated, often to the exclusion of full-length products. Therefore, some modifications to each of the major steps have been carried out.

4. MODIFICATIONS AND OPTIMINATION OF RACE

Reverse transcription

The most crucial step influencing the outcome of a RACE experiment is the generation of first-strand cDNA by reverse transcription of mRNA, especially for collecting full-length cDNA of 5'-ends. The reason is that the higher content of GC at the end of 5'-end of mRNA, which give rise to truncated fragment in reverse transcription . Besides high quality of mRNA,

an effective way is to raise the temperature of the reaction to destabilize mRNA secondary structure in use of rTth and TetZ.

Alternative to TdT tailing

In the original RACE protocols, homopolymer tailing strategy led to possibly internal priming and fail to clone full-length cDNA. Several groups have independently devised modifications of RACE methods which circumvent TdT-mediated tailing of the cDNA. These methods all incorporate the use of RNA ligase to covalently attach an anchor oligonuleotide to the 5'-end of the cDNA, such strategies thereby eliminate both the TdT tailing step and the need for anchor-primed second-strand cDNA synthesis.

Improvement of PCR Amplification

Although PCR technique has been improved greatly, some groups deal with PCR conditions such as fidelity increasing, suitable primer design, thermocycling parameters preferring and specificity improving. As a result of these, it is easily to achieve complete cDNA. Therefore, RACE is utilized in more fields.

5. NOVEL APPLICATIONS OF RACE

Although most RACE protocols have been designed with the cloning of full-length cDNA end as the goal, some investigators have modified the basic protocols to clone neighboring internal sequences. Fritz [9]devised a RACE to clone unknown neighboring internal sequences for use when the poly(A) tail is too distant to allow cloning by standard 3'RACE methodology; Struck and Collins [10] have used a similar method to clone unknown neighboring 5' sequences which involves using a specific 3'-end primer and a spurious 5'-end primer; Whitcomb have devised a method which they call random-primed/anchored-PCR to generate nested deletions from a target sequence which has been cloned into a plasmid vector. All of these have expanded the use of RACE.

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