

Random-primed cDNA synthesis facilitates the isolation of multiple 5'-cDNA ends by RACE

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The RACE (rapid amplification of cDNA ends) technique (1, 2) can be used to amplify 5'- and 3'-cDNA ends that derive from transcripts of low abundance. To isolate the 5' end of a specific cDNA (5' RACE), a small, anti-sense, transcript-specific oligonucleotide is used to prime first-strand cDNA synthesis. The specific first-strand cDNA is then purified, and polyadenylated using terminal deoxynucleotidyl transferase (TdT) and dATP. This synthetic poly(A) tract serves as the target for a poly(T)-containing primer during the subsequent amplification process (1). One drawback of this approach is that specifically-primed first-strand cDNA must be synthesized for each transcript under study; this is tedious when one is attempting to isolate different 5'-cDNA ends (e.g. those that encode different members of a multi-gene family) using the same RNA source. A second problem is that some specific primers may anneal inefficiently to the transcript of interest as a result of mRNA secondary structure. To overcome such difficulties, we have successfully used random-primed, polyadenylated, first-strand cDNA in combination with 5' RACE.

Random-primed first-strand cDNA synthesis was as follows. Either 20 µg of total RNA or 1 µg of poly(A)⁺ RNA was suspended in diethylpyrocarbonate (DEPC)-treated distilled H₂O, heated to 65°C for 5 min, then rapidly cooled on ice. To this was added: 1 µl (40 units) RNasin (Promega), 10 µl 5× reverse transcription buffer (500 mM Tris-HCl (pH 8.15 at 42°C), 600 mM KCl, 100 mM MgCl₂), 2.5 µl each dNTP (5 mM), 500 pmol random hexanucleotides (Pharmacia), 1 µl 1 M DTT, 1 µl (15 units) Rous associated virus 2 (RAV-2) reverse transcriptase (Amersham), and DEPC-treated distilled H₂O to a final volume of 50 µl. Incubation was at 42°C for 2 h. The cDNA was precipitated, after increasing the volume to 100 µl with distilled H₂O, by adding 10 µl 3 M sodium acetate (pH 4.8) and 60 µl propan-2-ol, and by incubating on ice for 15 min. The pellet was recovered by centrifugation, washed with 80% (v/v) ethanol and resuspended in 100 µl distilled H₂O. The precipitation and washing step was then repeated twice; this purifies the first-strand cDNA from the excess nucleotides.

For polyadenylation, the cDNA was resuspended in distilled H₂O (21 µl), boiled for 2 min, and rapidly cooled on ice. The following components were then added: 1 µl 6 mM dATP, 6 µl 5× tailing buffer (500 mM potassium cacodylate (pH 7.2), 10 mM CoCl₂, 1 mM DTT), and 2 µl (15 units) TdT (Amersham). Incubation was at 37°C for 1 h. Amplification of 5'-cDNA ends can then be performed directly on a small aliquot (~1%) of the polyadenylated cDNA essentially as described (2).

Using random-primed, polyadenylated, first-strand cDNA in combination with the RACE protocol, we have cloned, and subsequently characterized, 5'-cDNA ends of up to 800 bp in length that correspond to insect receptor transcripts of very low abundance (Figure 1). The modification described here aids the rapid isolation of multiple 5'-cDNA ends from a single RNA source.

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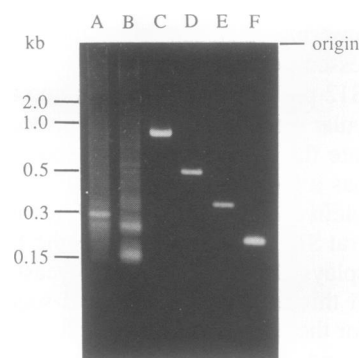


Figure 1. Agarose gel electrophoretic analysis of 5'-RACE products that encode the N-terminal portion of a locust glutamate receptor subunit. These cDNAs were generated from *Schistocerca gregaria* adult muscle total RNA. Lanes A and B: Unfractionated 5'-RACE products amplified from specifically-primed and random-primed first-strand cDNA, respectively. Lanes C to F: Overlapping cDNAs of various lengths isolated from 5'-RACE products that were amplified from random-primed first-strand cDNA (lane B). These cDNAs were size-selected and cloned into M13 vectors; insert lengths were determined by PCR (3). The relative positions of size markers are shown in kb.