Efficient double stranded sequencing of cDNA clones containing long poly(A) tails using anchored poly(dT) primers

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Sequencing double stranded DNA templates has become a common and efficient procedure (1) for rapidly obtaining sequence data while avoiding preparation of single stranded DNA. Here we report the applicability of this procedure to sequencing cDNA clones containing long stretches of poly(A). Double stranded templates of cDNAs containing long poly(A) tracts are difficult to sequence with vector primers (e.g. universal M13) which anneal downstream of the poly(A) tail. Sequencing with these primers results in a long poly(T) ladder followed by a sequence which is difficult to read (Fig. 1). In an attempt to solve this problem we synthesized three primers which contain (dT)₁₇ and either (dA) or (dC) or (dG) at the 3' end. We reasoned that the presence of these three bases at the 3' end would 'anchor' the primers at the upstream end of the poly(A) tail and allow sequencing of the region immediately upstream of the poly(A) region.

Anchored primers were synthesized on an Applied Biosystems (ABI) 391 DNA synthesizer and used after purification on Oligonucleotide Purification Cartridges (ABI). For sequencing with anchored primers, 5–10 µg of plasmid DNA was denatured in a total volume of 50 µl containing 0.2 M sodium hydroxide and 0.16 mM EDTA by incubation at 65°C for 10 minutes. The three poly(dT) anchored primers (2 pmol of each) were added and the mixture immediately placed on ice. The solution was then neutralized by the addition of 5 µl of 5 M ammonium acetate pH 7.0. The DNA was precipitated by addition of 150 µl of cold 95% ethanol and the pellet washed twice with cold 70% ethanol. The pellet was dried for 5 minutes and then resuspended in 1×sequencing buffer (1× = 40 mM Tris–HCl pH 7.5, 20 mM MgCl₂, 50 mM NaCl). Primers were annealed by heating the solution for 2 minutes at 65°C followed by slow cooling to room temperature. Sequencing reactions, using modified T7 DNA polymerase (Sequenase, United States Biochemicals), were then carried out using [³²P]α-dATP (> 1000 Ci/mmol) according to the protocol supplied with the Sequenase kit. Under these conditions over 300 bp of readable sequence could be obtained (Fig. 1). We have applied this approach to several other poly(A)-containing cDNA clones with similar results. Sequencing of the opposite strand of these cDNAs using insert-specific primers verified that the sequences obtained with the anchored primers occurred directly upstream of the poly(A) region (data not shown).

The ability to directly obtain sequence immediately upstream

from the poly(A) tail of cDNAs, as demonstrated here, should be of particular importance to large scale efforts to generate sequence-tagged sites (STSs) (2) from cDNAs (3).

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Figure 1. Comparison of sequencing through poly(A) using either the M13 universal primer or anchored poly(dT) primers. A cDNA clone selected from a λZAPII human brain cDNA library was first autoexcised into Bluescript plasmid according to the protocol recommended by the supplier (Stratagene). After preparation of plasmid DNA by standard procedures (4) the cDNA insert was sequenced using either the M13 universal sequencing primer (Lane 1) or an equimolar mixture of the three poly(dT) anchored primers (Lane 2). Sequencing reaction products were resolved on a 6% polyacrylamide/urea gel. The sequences shown in the Figure begin approximately 50 bp upstream of the corresponding primers.