Sequencing Through GC-rich and AT-rich Sequences with the SequiTherm™ EXCEL™ II DNA Sequencing Kit

Meena Kumari and Antje Anji, Dept. of Pharmacology, Univ. of Texas Health Science Center

Introduction

Messenger RNA stability, an important control mechanism of gene expression, is a highly regulated process. The majority of mRNA stability determinants have been located within the 3′-UnTranslated Region (UTR) of an mRNA. To locate stability determinants for a brain-specific receptor protein being studied in our lab, we first generated a full-length cDNA clone. Since the sequence of the UTR of the gene of interest is unknown, we sequenced that part of the cDNA. Here we present a method to sequence a GC-rich double-stranded DNA template using the SequiTherm™ EXCEL™ II DNA Sequencing Kit.

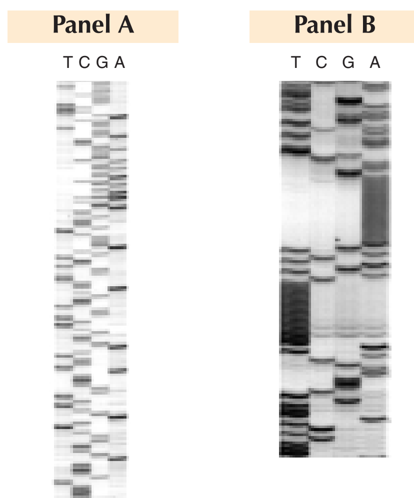

Figure 1. DNA regions from cDNA clones with high GC- (Panel A) or AT- (Panel B) content are readily sequenced by SequiTherm™ EXCEL™ II DNA Polymerase.

Methods

The UTR of the gene of interest was amplified by RACE and the products were subcloned. Plasmid miniprep DNA was used as sequencing template. Primers, both universal and gene-specific, were 5′-end-labeled with [γ-^{33}P]ATP. Sequencing premix was prepared as follows: 300 fmoles DNA template was vacuum dried, and resuspended in 6 µl autoclaved milliQ water. DNA was then mixed with 6 µl (12 pmoles) of ^{33}P-labeled primer, 8.5 µl of SequiTherm EXCEL II sequencing buffer and stored on ice for 15 minutes before adding 1 µl (5 Units) of SequiTherm EXCEL II DNA polymerase. Five microliters of the sequencing premix were added to each of four tubes containing the respective SequiTherm EXCEL II termination mix (A= 2.4 µl; C= 3 µl; G= 3 µl and T= 2.4 µl). The cycle DNA sequencing reaction was performed as follows: Step 1 = 5 minutes at 95ºC x 1 cycle; Step 2 = 30 seconds at 95ºC, 50 seconds at 68ºC, 60 seconds at 72ºC x 30 cycles; Step 3 = soak at 6ºC. At the end of the sequencing reaction, 3 µl of Stop/Loading buffer was added to each of the tubes and each tube was denatured at 85ºC for 3 minutes, before loading samples (1.5 – 4.5 µl) on a 5% denaturing polyacrylamide gel. Following electrophoresis, the gel was fixed, dried and then exposed to Kodak Biomax X-ray film overnight at room temperature for 24 hours.

Results and Discussion

Using the SequiTherm EXCEL II Kit and the sequencing conditions described here, we were able to generate sequence data beginning very near the 3′-end of the primers. Typically an electrophoretic run resulted in a separation of a easily readable sequence of at least 300 nucleotides. Analysis of the sequences generated revealed regions of very high (74%) GC-content (Figure 1A). The SequiTherm EXCEL II DNA Polymerase successfully read through these regions without any noticeable "stops" or bands in all four lanes as can occur with some polymerases in high GC regions.

While sequencing other UTR clones we found that some clones were unique and not related to our gene of interest. One such sequence is shown in Figure 1B. This sequence has a high AT-content which again was easily sequenced by the SequiTherm EXCEL II DNA Polymerase. Taken together our sequence data (shown and not shown) indicates that the SequiTherm EXCEL II Kit is an excellent kit for sequencing both GC-rich and AT-rich DNA templates.

Acknowledgment

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