Improved QPCR Analysis Following Storage in RNAstable™

Radha Gopal, V.Sc. and Fernando P. Monroy, Ph.D.
Department of Biological Sciences, Northern Arizona University

Introduction:

RNAstable™ is a novel RNA preservation product designed to protect RNA samples from degradation during storage or shipment at ambient temperatures. RNAstable is formulated based on the natural principles of a biological mechanism called anhydrobiosis (meaning "life without water"). Anhydrobiotic organisms protect their cellular contents and structures for survival while in a dried state for over 100 years and can be revived with simple rehydration. RNAstable was designed to mimic these unique characteristics through synthetic chemistry to stabilize RNA dry at ambient temperatures. The data presented demonstrate successful amplification with quantitative RT-PCR (qRT-PCR), using a RNA template that was stored dry in RNAstable for 4 weeks at room temperature. Samples recovered by rehydration were used directly in reactions without further purification and exhibited no inhibition or loss of activity. This innovative technology offers significant cost and energy savings as an easy-to-use alternative to conventional cold sample storage and shipment.

Materials and Methods:

A mouse intestinal epithelial cell line (MODE-K) was grown to confluence in a T-75 tissue culture flask in DMEM medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal calf serum (HyClone, Logan, UT), 100 U of penicillin/ml, 50 µM 2-mercaptoethanol, and 0.1 mg/ml of streptomycin (Invitrogen). Cells were harvested after brief incubation in 0.25% trypsin, centrifuged at 800 rpm for 10 min, and the pellet resuspended in 1 ml sterile PBS. RNeasy® (Qiagen, Valencia, CA) was used to isolate total RNA following the manufacturer's instructions. RNA concentration was determined by spectrophotometry ($A_{260/280}$) and RNA was distributed equally into tubes for storage either dry at room temperature in RNAstable or at -80°C as control samples. RNA samples (10 µl aliquots) applied into tubes containing RNAstable were dried in a vacuum concentrator for 1.5 h after which tubes were capped and stored at room temperature for 1 and 4 weeks.

A total of 2.0 µg of purified total mRNA was reversed transcribed at each time point using the First Strand cDNA Synthesis Kit (Fermentas, Glen Burnie, MD) which utilizes the recombinant M-MuLV Reverse Transcriptase. A total of 500 ng of cDNA was amplified using the SYBR Green master mix (SuperArray, Frederick, MD) on a Bio-Rad iCycler. Relative expression was calculated using the standard curve approach in which known concentrations of gel purified products are used to determine the copy number of the unknown samples. The following primers were used to amplify mouse GAPDH: forward 5'-CGTCGTGGACATGACGTG and reverse 5'-CCTGCTTCACCACCTTCTTG.

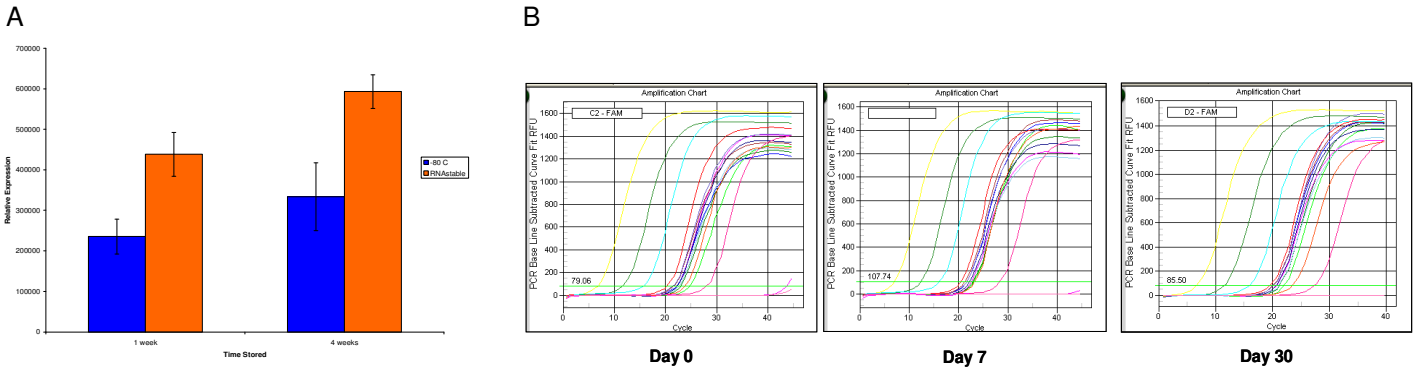

Figure 1: Aliquots of total RNA were stored at either -80°C or at room temperature in RNAstable for up to 30 days. (**A**) Comparison of relative gene expression from samples stored at -80°C (blue) or in RNAstable (orange). (**C**) An aliquot (500 ng) of cDNA was amplified for each storage condition at days 0, 7 and 30 using real time PCR and the results are overlaid on the graphs.

Results and Discussion:

We consistently found higher expression (copy number) in the RNA samples stored in RNAstable. It is clear that RNA integrity of samples stored at -80°C was affected when compared to those placed in RNAstable. It is possible that during our initial RNA isolation, samples destined for freezer storage were damaged while being kept at -20°C during the 1.5 h time period in which RNAstable samples were being dried in the vacuum concentrator. After this time period, freezer control samples were moved from -20°C to -80°C for long term storage, while the samples in RNAstable were maintained at room temperature. Regardless, the decrease in RNA integrity from the control freezer stored samples resulted in lower amplification of target product. This was not the case for RNA protected in RNAstable, indicating its value in maintaining RNA integrity even during the initial concentration of samples. Rehydrated samples used directly in reactions without further purification did not exhibit any interference or inhibition during qRT-PCR analysis.