Microarray Analysis of RNA in RNAstable™

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Introduction:

RNAstable™ is a novel RNA preservation product developed to protect RNA samples from degradation during storage or shipment at ambient temperatures. RNAstable is formulated by combining extremophile biology (long-term survival in extremely dry environments in an arrested life form) and synthetic chemistry. Anhydrobiosis (meaning "life without water") allows some organisms to protect their DNA, RNA, proteins, membranes and cellular systems for survival; they are revived by simple rehydration. RNAstable was designed to mimic these unique characteristics to stabilize RNA at ambient temperatures.

The data presented demonstrate that RNA stored in RNAstable can be used successfully for microarray analysis with the Affymetrix™ Human Genome HG-U133 Plus 2.0 array platform to obtain gene expression profiles. Samples recovered by rehydration were used directly for analysis without further purification and exhibited no inhibition or loss of activity. The quality of the recovered RNA was confirmed using an Agilent 2100 bioanalyzer and was identical to control samples. Comparison of gene expression profiles indicate no significant differences between control freezer stored samples and those kept at room temperature protected in RNAstable.

Materials and Methods:

Sample Preparation and Storage in RNAstable:

Total RNA was isolated from human fetal cartilage tissues as described in Krakow *et al.* (*Mol Genet Metab* 2003, 79:34-42) using the TRIzol® isolation method. Total RNA was resuspended in RNase-free water and stored at -80℃ until ready for use. An aliquot of total RNA was applied to RNAstable in the provided 1.5 ml microfuge tube (Biomatrica catalog #933221-001) and dried in a SpeedVac® for 40 min at room temperature. RNA was then left for 1 day at room temperature, while a separate aliquot was stored at -80℃ for control purposes. The quantity and quality of all the stored RNA samples were then analyzed using an NanoDrop® ND-1000 spectrophotometer and Agilent 2100 bioanalyzer (Fig 1.), respectively.

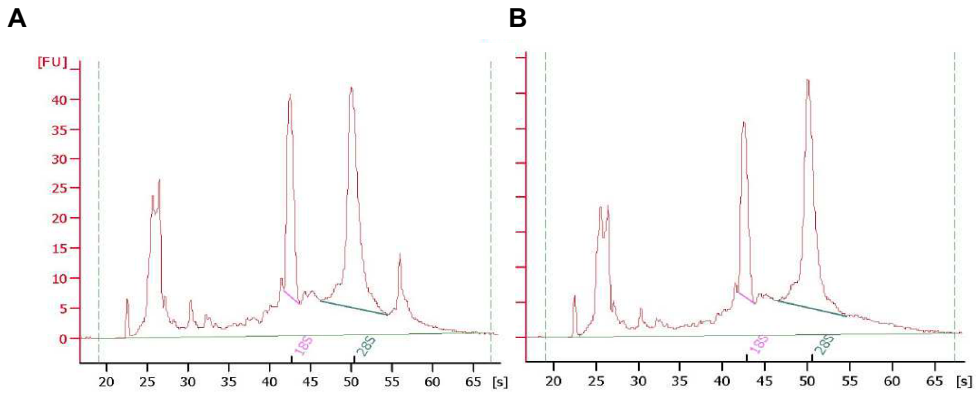

Figure 1: Agilent 2100 Bioanalyzer RNA profiles show no difference after drying and storage with Biomatrica at room temperature as compared to conventional storage at -80℃. Note that the 5S, 18S and 28S peaks are intact. (**A**) Profiles of total RNA derived from human fetal cartilage samples stored at -80℃ or (**B**) at room temperature protected in RNAstable for 1 day, and analyzed using an Agilent 2100 bioanalyzer.

Microarray analysis of RNA stored in RNAstable:

Whole genome microarrays were used to test for any degradation of RNA in Biomatrica stored samples as compared to freezer stored samples. Microarrays are more sensitive to degradation than qRT-PCR and enable deeper analysis of the transcriptome. For technical and statistical purposes, two aliquots of each (RNAstable and freezer stored RNA samples) were arrayed on Human Genome Affymetrix HG-U133 Plus 2.0 arrays. This platform was chosen for its complete coverage of the Human Genome U133 and its ability to simultaneously measure approximately >47,000 transcripts. Statistical analysis, including data normalization and transformation, was performed using the BioConductor R package. Specifically, the MAS 5.0 algorithm was used for qualitative analysis (*e.g.* absence or presence of a transcript), while the Plier algorithm was used for quantitative analysis.

QC statistic based on ratio of intact (△) actin and (o) GADPH transcripts

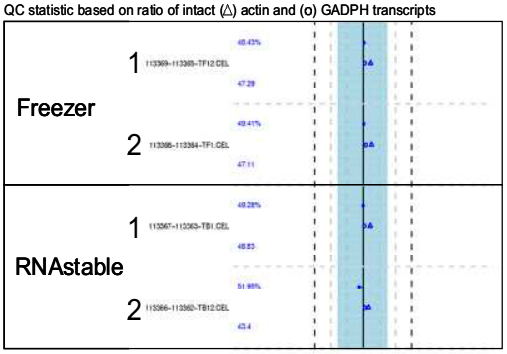

Figure 2: Whole genome microarray analysis suggested no difference in downstream biochemical applications (*e.g. in vitro* transcription) or degradation when stored using RNAstable compared to freezer stored samples. Quality control statistics using the 5'/3' ratios of actin and *GAPDH* transcripts are plotted and indicate identical results between the two storage methods.

Calls	FROZEN	FROZEN	Frozen	BIOMATRICA	BIOMATRICA	BIOMATRICA
Number Present:	0.494	0.484	0.489	0.5065	0.52	0.493
Number Absent:	0.491	0.5	0.4955	0.4785	0.465	0.492
Number Marginal:	0.014	0.015	0.0145	0.0155	0.016	0.015
Average Signal (P):	8234.9	8465.7	8350.3	7867.25	7527.7	8206.8
Average Signal (A):	249.4	253.5	251.45	242.95	236.7	249.2
Average Signal (M):	746.4	756.9	751.65	703.65	656	751.3
Average Signal (All):	4202.7	4238.6	4220.65	4104.35	4030.9	4177.8

Degredation of Housekeeping Controls:	FROZEN				BIOMATRICA	
Probe Set	Sig(3'/5')	Sig(3'/5')	Frozen	BIOMATRICA	Sig(3'/5')	Sig(3'/5')
AFFX-HUMISGF3A/M97935	9.86	8.96	9.41	7.515	6.77	8.26
AFFX-HUMRGE/M10098	0.38	0.57	0.475	0.29	0.3	0.28
AFFX-HUMGAPDH/M33197	1.13	1.07	1.1	1.06	1.07	1.05
AFFX-HSAC07/X00351	1.42	1.38	1.4	1.3	1.25	1.35
AFFX-M27830	0.01	0.02	0.015	0.01	0.01	0.01

No significant difference between samples was observed

Table 1: The number of present and absent calls and the average signal intensities did not reveal any significant differences between samples stored frozen or those maintained at room temperature in RNAstable (Biomatrica). Individual probesets were further assessed for concerted changes (*e.g.* absent to present or vice versa) between storage conditions. Among all probesets assessed, only one probeset behaved differently between the two RNA storage conditions (data not shown).

Results and Discussion:

There were no qualitative differences in RNA expression measured between the control samples stored at -80℃ and samples stored at room temperature in RNAstable as determined using the Agilent bioanalyzer and whole genome microarray expression analysis. Agilent RNA profiles confirmed that samples stored in RNAstable had no increased degradation when compared to the freezer stored controls. In fact, the optimal RNA ratio appeared better conserved in samples stored in RNAstable stored sample compared to control samples stored frozen. In addition, the microarray data were querried to reveal differences in the absence or presence of a transcript. Remarkably, the absolute expression of only one probeset differred in the analysis, further suggesting that there was no qualitative difference in downstream biochemical reactions or degradation between the two sample storage conditions.

RNA expression profiles from RNAstable stored samples were then compared in an unsupervised fashion to frozen samples to determine if there were any quantitative or qualitative differences between storage conditions. After filtering out genes which varied by more than three-fold among two or more samples, two-way hierarchical clustering between genes and arrays using Pearson correlation was employed (data not shown). This analysis revealed no correlation between gene expression and storage condition, suggesting there is no difference in genome-wide transcript population between freezer storage and storage in RNAstable at room temperature.

Conclusions:

These global gene expression profiling results indicate that RNAstable does not exhibit any interference or inhibition of downstream biochemical reactions (*e.g. in vitro* transcription) when used for storage and stabilization of total RNA. Recovered RNA can be used directly without the need for further purification in a variety of downstream applications including quantative RT-PCR, transcription, agarose gel electrophoresis and gene expression analysis.

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