Short Technical Reports

Assessing a novel room-temperature RNA storage medium for compatibility in microarray gene expression analysis

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RNA integrity is a critical factor in obtaining meaningful gene expression data. Current methodologies rely on maintaining samples in cold environments during collection, transport, processing, and storage procedures, which are also extremely time-sensitive. Several RNA storage products are commercially available to help prevent degradation during the handling and storage steps; however, samples must be kept cold for optimal protection. We have evaluated a novel RNA storage medium based on anhydrobiosis for stabilizing and protecting samples from degradation at room temperature that are intended for use in microarray analysis. Samples were stored dry at room temperature for various time periods to assess any degradation or loss of activity as compared with frozen control samples. Recovered samples were used directly for analysis without further purification and exhibited no interference or inhibition in downstream applications. Comparison of gene expression profiles indicate no significant differences between freezer-stored control samples and those kept at room temperature protected in the RNA storage medium. The quality of recovered RNA was confirmed using spectrophotometry and Bioanalyzer analysis and was identical to control samples. The ability to stabilize RNA samples at ambient temperatures for extended time periods will have tremendous use, particularly for sample shipment to core facilities.

Introduction

The use of microarray technology to measure changes in gene expression profiles has become a standard tool for studying biological models of disease, growth, and development, and is increasingly being used in the field of clinical research. While technology advances continue to increase the sensitivity of expression profiling assays such as quantitative real-time PCR and microarray analysis, the fundamental baseline for generating quality data rests on the quality and integrity of the RNA sample.

Special care is needed when working with RNA due to its chemical instability and the ubiquitous presence of RNases in the laboratory. Commercially available products, such as RNAlater (Ambion, Austin, TX, USA), have enabled successful preservation of tissues and cells for long-term storage at cold or freezing temperatures prior to RNA purification (1–3). Room-temperature storage (18–25°C) in RNAlater is possible for up to 7 days according to manufacturer's instructions, although significant mRNA degradation was detected after 3 days of room-temperature storage of rat liver samples soaked in RNAlater (2). Purified RNA samples are handled minimally and kept cold at all times unless otherwise directed. Samples are typically stored frozen (-20°C, -80°C, or under liquid nitrogen) in RNase-free solutions (e.g., DEPC-treated water, TE buffer or other commercially available RNA storage solutions) and thawed on ice only when needed. Multiple freeze-thaw cycles and prolonged exposure to increased temperatures are avoided as these conditions serve to promote degradation of precious and labile RNA samples. Despite all the precautions taken to maintain what is typically referred to as cold chain logistics, the integrity of a sample is often compromised using current cold storage methodologies, particularly during long-term storage or sample transport, where shipment delays and suboptimal packaging are a common occurrence. There is a critical need for technologies providing simple room-temperature storage of biological samples that do not require cold chain logistics or complicated sample recovery protocols.

A novel storage medium designed specifically to preserve and protect purified total RNA samples stored dry at room temperatures is commercially available. RNAstable (Biomatrica, San Diego, CA, USA) is a synthetic matrix that was developed based on the principle of anhydrobiosis, a biological mechanism employed by some multicellular organisms that enables their survival while dry for >100 years (4). The matrix forms a thermostable barrier around RNA during the drying process to protect samples from degradation during storage at ambient temperatures. Samples are ready for immediate analysis following a single rehydration step, eliminating the need for further downstream purification. We have evaluated the use of RNAstable for storage of purified total RNA samples derived from various human tissues for compatibility in microarray analysis for gene expression profiling. The quality of the RNA was assessed by RNA Integrity Number (RIN) score [on an Agilent 2100 Bioanalyzer (Palo Alto, CA, USA)], yield of total RNA, $OD_{260/280}$ ratio, and by the microarray QC parameters of percent present and 3′/5′ ratios of GAPDH and β-actin probe sets on an Affymetrix gene expression array (GLYCOv3 array; Consortium for Functional Glycomics). Results indicate that samples stored for ≤5 weeks at room temperature while protected in RNAstable in a desiccating environment are indistinguishable from control samples stored frozen at -80°C. Samples rehydrated from storage in RNAstable were used directly without further purification and did not

Table 1. Recovery and integrity of RNA stored in RNAstable

RNA Sample	Storage Condition	ng/µL	$OD_{260/280}$	RIN
Human liver total RNA	*RT for 4 weeks (RNAstable)	219 ± 10	2.02 ± 0.03	9.70 ± 0.00
	-80°C for 4 weeks (RNase-free water)	217 ± 9	2.05 ± 0.01	9.73 ± 0.06
Human kidney total RNA	RT for 1 week; -80°C for 4 weeks (RNAstable)	243 ± 15	2.00 ± 0.05	8.08 ± 0.15
	-80°C for 5 weeks (RNase-free water)	296 ± 7	1.98 ± 0.01	7.98 ± 0.15

*RT, room temperature.

Table 2. Glyco_v3 microarray analysis of human liver total RNA stored in RNAstable

Storage Condition	Background	Noise	% Present	GADPH (3′/5′ ratio)	β-actin (3′/5′ ratio)
RNAstable (RT for 4 weeks)	37.3 ± 1.6	1.6 ± 0.2	59.3 ± 1.0	1.14 ± 0.06	4.22 ± 0.71
Control (-80°C for 4 weeks)	37.4 ± 1.6	1.7 ± 0.1	59.4 ± 1.3	1.10 ± 0.06	4.10 ± 0.20

[a]Glyco_v3 microarray is a custom GeneChip expression array built by Affymetrix for the Consortium of Functional Glycomics; $n = 3$.

exhibit any interference or inhibition during microarray analysis. Use of RNAstable for room-temperature storage of purified RNA can reduce reliance on current cold storage methodologies. The storage medium is particularly suited for sample transport, as unexpected delays will not damage the RNA. Samples can be prepared with minimal effort and shipped without expedited delivery fees or bulky dry-ice containers. Maintaining a controlled humidity (i.e., desiccating) environment for optimal stabilization and protection of RNA is easily achieved by storing dried samples in packaging that includes a desiccant pack supplied by the manufacturer. Such applications will have particular use for sample transport to core array facilities.

Materials and Methods

RNA preparation

Purified human liver and kidney total RNA (100 µg) were purchased from Ambion and diluted to 250 ng/µL. The quality of the RNA prior to any treatment was assessed using an Agilent 2100 Bioanalyzer and the RIN was determined to be 9.6 and 7.9 for the human liver and kidney total RNA, respectively. Aliquots (20 µL) were applied directly into 1.5-mL tubes containing RNAstable (supplied with the kit) and dried in a Savant Integrated SpeedVac System ISS110 vacuum concentrator (Instrument Inc, Holbrook, NY, USA) without heat for 30 min following manufacturer's instructions prior to storage at room temperature for various time periods. Dried samples were then stored at room temperature on the benchtop inside a sealed-moisture barrier bag including a desiccant pack (both are included with the kit), following manufacturer's guidelines. Aliquots (20 µL) used for frozen control samples were placed into 1.5-mL Eppendorf tubes (Cat. no. 1615-5510, USA Scientific Inc, Orlando, FL, USA) and stored at -80°C until ready for use. Samples were recovered from storage in RNAstable by rehydrating with 20 µL RNase-free water and used directly for analysis without further purification. RNA quantification was determined by spectrophotometry using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc, Wilmington, DE, USA). The $OD_{260/280}$ ratio was used to evaluate the purity of the nucleic acid samples and the quality of the extracted total RNA was determined with the Agilent Bioanalyzer.

For the drying time experiment, total RNA was prepared from mouse spleen using the RNeasy Mini-Kit (Qiagen, Valencia, CA, USA). Purified mouse spleen RNA of concentration 287 ng/µL was then applied as 25-µL aliquots into RNAstable storage tubes. Samples were dried for 60, 90 and 120 min in the Savant Integrated SpeedVac System ISS110 with the drying rate set to 'low.'

Microarray Analysis

RNA from 3 replicate preparations of samples (500 ng) stored frozen at -80°C (control) or dry at room temperature in RNAstable were labeled using MessageAmp II-Biotin Enhanced Kit (Applied Biosystems, Foster City, CA, USA) following manufacturer's instructions and hybridized to the GLYCOv3 array (Consortium for Functional Glycomics). Arrays were scanned using Affymetrix ScanArray 3000 and standard Affymetrix protocols (www.affymetrix.com). Present and absent calls were determined with the Affymetrix algorithm using default settings.

Results and Discussion

To evaluate the use of RNAstable for storage of purified total RNA samples for compatibility in microarray analysis, triplicate samples of total RNA purified from human liver (Ambion) were stored dry in RNAstable on the laboratory bench top at room temperature for 4 weeks prior to analysis and comparison to frozen control samples stored at -80°C. Samples in RNAstable were recovered by adding RNase-free water directly to the dried sample; the rehydrated sample was then used for analysis without further purification. Control samples were thawed on ice immediately prior to analysis. The $OD_{260/280}$ ratio was used to evaluate the purity of recovered RNA samples and average values were determined to be 2.02 ± 0.03 and 2.05 ± 0.01 for samples recovered either from RNAstable or frozen storage, respectively (Table 1). The RNA samples were assessed after storage by spectrophotometry and using an Agilent Bioanalyzer to generate a RIN value.

Results of triplicate samples analyzed for each storage condition indicate that the yield (ng/µL) of RNA recovered following storage in RNAstable is comparable to that of frozen control samples, even after 4 weeks of storage on the bench top at room temperature (Table 1). The average concentration of RNA recovered in RNAstable was 219 ± 10 ng/µL, which was comparable to the frozen control (217 ± 9 ng/µL) stored for

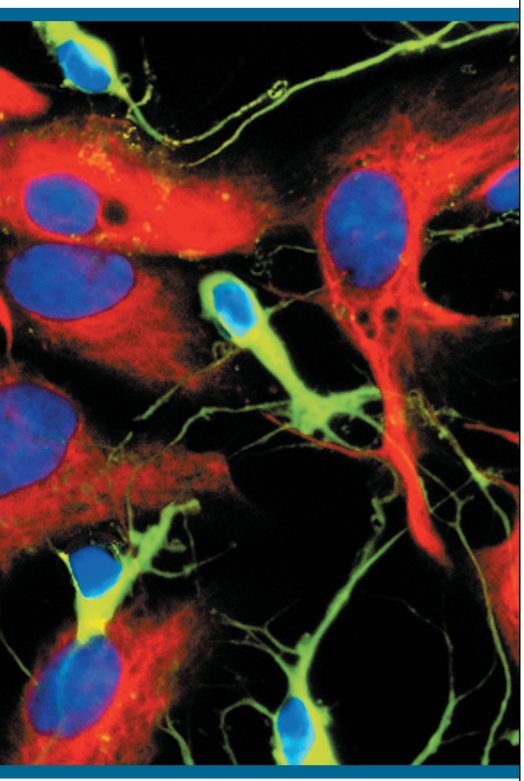

Table 3. Effect of initial drying time on the stability of mouse spleen total RNA stored in RNAstable

	Drying Times (min)[a]			
	0	60	90	120
Concentration (ng/µL)	287.92 ± 0.0	231.20 ± 1.03	232.81 ± 8.16	235.55 ± 8.97
RIN	7.1 ± 0.0	7.3 ± 0.1	7.1 ± 0.0	7.2 ± 0.2

[a] $n = 3$ for each time point.

the same time period. We also assessed the effect of exposure to extremely low temperatures on sample stability by storing human kidney total RNA samples in RNAstable at -80°C for 4 weeks after initial storage at room temperature for 1 week. Results indicate that the protective properties of RNAstable are not affected by exposure to extremely low temperatures and were comparable to control samples stored frozen (Table 1).

The rehydrated human liver total RNA samples were assessed for their compatibility and usefulness in microarray gene expression analysis. Table 2 shows the standard Affymetrix GeneChip expression array QC parameters including: background, noise, % present, and the 3′/5′ ratios for GAPDH and β-actin. Results indicate nearly identical values for control samples stored frozen at -80°C and dried samples protected in RNAstable at room temperature.

As storage of RNA in RNAstable requires drying of the sample after application into the matrix, we investigated whether the drying time affects sample recovery. Aliquots (25 µL) of total RNA purified from mouse spleen (287 ng/µL) were applied into tubes containing RNAstable in triplicate and dried for 60, 90, and 120 min using a vacuum concentrator. Samples were rehydrated immediately after drying with 25 µL RNase-free water and the concentrations and RIN scores were determined. Time 0 refers to the stock RNA sample prior to application into tubes containing RNAstable. These results show that the RNAstable storage system works well under a range of drying times and protects the RNA sample from over-drying under vacuum and low heat (Table 3).

Based on our results, we have found that room-temperature storage of RNA in RNAstable for ≤4 weeks is suitable for samples destined for microarray analysis, and provides an alternative to conventional cold storage and transport procedures currently used. These results further indicate that samples can be used directly for microarray analysis applications without needing to remove the matrix from the sample. Eliminating extraneous purification protocols saves time and also reduces sample loss typically associated with traditional ethanol precipitation or column purification methods. Furthermore, since the sample can be rehydrated in any desired volume, storage of RNA dry in RNAstable offers a convenient method for concentrating samples while also protecting them from degradation. Moreover, excessive drying had no effect on subsequent sample rehydration. We therefore recommend the use of a vacuum concentrator when preparing samples for storage in RNAstable as complete drying is critical for optimal formation of the thermostable barrier, in addition to maintaining dried samples in a desiccating environment during storage and shipment. The ability to store purified total RNA samples for long time periods at room temperature has significant advantages and applications for molecular biology research, particularly in the area of sample transport. RNAstable offers a convenient alternative for transporting samples dry at ambient temperatures without having to rely on cold storage to prevent RNA degradation.

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