

# Application Forum

## Ambient temperature stabilization of purified RNA in GenTegra™ for use in Affymetrix Human Exon 1.0 ST arrays

M. G. Barnes<sup>1,2</sup>, M. Tsoras<sup>1</sup>, S. D. Thompson<sup>1</sup>, H. Martinez<sup>3</sup>, B. Iverson<sup>3</sup> and R. Nuñez<sup>3</sup>

<sup>1</sup>Cincinnati Children's Hospital Medical Center, Division of Rheumatology, Cincinnati, OH, USA

<sup>2</sup>Cincinnati Children's Hospital Medical Center, Cincinnati Biobank Core Facility, Cincinnati, OH, USA

<sup>3</sup>GenVault Corporation, Carlsbad, CA, USA

### Introduction

RNA derived from peripheral blood mononuclear cells (PBMCs) is frequently used for gene expression analysis in human disease research. Once purified from cells or tissue, RNA stability must be maintained to avoid the loss of valuable samples. Preventing degradation of RNA, which is primarily induced by RNases that co-purify with nucleic acids or are introduced from the environment, is a major challenge, as is protecting purified RNA during long-term storage and transport. The Pediatric Rheumatology Tissue Repository and the Cincinnati Biobank Core Facility at the Cincinnati Children's Hospital Medical Center face these challenges when working with RNA purified from Ficoll-isolated PBMCs (1-4). The use of purified RNA is an important part of the ongoing research to understand the biology and pathogenesis of juvenile idiopathic arthritis, and other immunologic diseases of childhood.

### A simple solution for RNA stabilization

A new product for the stabilization of purified RNA was recently introduced by GenVault Corporation. GenTegra™ RNA is an inert chemical matrix that inactivates trace RNase and protects RNA samples from hydrolysis and oxidation, enabling long-term storage and transport of purified RNA in the dry state at ambient temperature, and providing an added level of stability for RNA in the liquid state during sample handling. GenTegra™ is provided in ready-to-use aliquots (Figure 1).

### Materials and Methods

Aliquots containing 20 µL RNA purified from PBMCs were applied to GenTegra™ tubes or identical tubes without GenTegra™ and stored at 25°C or 37°C for 24 h. Additional sets of RNA aliquots were dried and stored for 2 weeks at 25°C or 56°C, then rehydrated in 20 µL water. Samples retained at -80°C served as a control. RNA was quantitated using a NanoDrop 1000 (ThermoScientific, Wilmington, DE, USA). RNA integrity number (RIN) was obtained using



**Figure 1.** GenTegra™ RNA 1.5-mL screw cap tubes (left) and 0.3-mL cluster tubes (right) contain an inert chemical matrix that stabilizes purified RNA at ambient temperature.

**Table 1.** RIN of samples stored in the liquid state for 24 h and in the dry state for 2 weeks at various temperatures in the presence or absence of GenTegra™.

Sample ID	Storage conditions for RNA in liquid state for 24 hours				
	-80°C	25°C		37°C	
	Frozen control*	GenTegra	No protection	GenTegra	No protection
1	9.8	9.3	9.5	8.9	2.2
2	9.8	9.5	9.0	9.2	---
3	9.5	9.5	9.6	9.1	---
4	9.7	9.7	9.5	9.4	---
Median	9.6	9.5	9.5	9.2	---
Sample ID	Storage conditions for RNA in Dry State for 2 weeks				
	-80°C	25°C		56°C	
	Frozen control*	GenTegra	No protection	GenTegra	No protection
1	9.8	9.5	2.4	8.7	3.8
2	9.7	9.7	8.4	8.8	3.1
3	9.8	9.6	8.7	8.3	2.0
4	10.0	9.6	9.1	8.6	3.6
Median	9.8	9.6	8.6	8.7	3.4

\*Average expression of duplicate samples for controls only. ---, no RIN generated due to extensive degradation.

the RNA 6000 Nano Chip on the Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). RNA (100 ng) was labeled using NuGEN Ovation WGA (NuGEN Technologies, San Carlos, CA, USA) and cDNA produced. Labeled samples

Table 2. Correlation of raw expression values.

Donor #	Temp	Array ID	Temp	56°C	-80°C	56°C	-80°C	56°C	-80°C	56°C	-80°C
			1	2	3	4	5	6	7	8	
1	56°C	1	1	0.99	0.98	0.98	0.98	0.98	0.98	0.98	0.98
	-80°C	2	0.99	1	0.98	0.98	0.98	0.98	0.98	0.98	0.98
2	56°C	3	0.98	0.98	1	0.99	0.99	0.99	0.99	0.99	0.99
	-80°C	4	0.98	0.98	0.99	1	0.98	0.99	0.99	0.99	0.99
3	56°C	5	0.98	0.98	0.99	0.98	1	0.99	0.99	0.99	0.99
	-80°C	6	0.98	0.98	0.99	0.99	0.99	1	0.99	0.99	0.99
4	56°C	7	0.98	0.98	0.99	0.99	0.99	0.99	1	0.99	0.99
	-80°C	8	0.98	0.99	0.99	0.99	0.98	0.99	0.99	1	0.99

were hybridized to Affymetrix Human Exon 1.0 ST arrays. Array data were imported into Affymetrix Expression Console and GeneSpring GX 10 (Agilent Technologies, Palo Alto, CA, USA) and pre-processed using robust multichip averaging (RMA) (5). The percentage of genes called “present” was determined and used as a surrogate to measure interference with labeling or probing procedures.

## Results

Table 1 summarizes the RIN of RNA samples stored at various temperatures in the presence or absence of GenTegra™ for 24 h in the liquid state, or for 2 weeks in the dry state. Compared with control samples stored at -80°C, there was no difference in RNA integrity for any of the samples stored in the liquid state at 25°C for 24 h. However, in unprotected samples, significant degradation was observed following 24-h liquid storage at 37°C, while samples stored in the presence of GenTegra™ showed only a slight decrease in RIN (0.4 units difference in median values) compared with frozen controls.

The RIN of samples stored dry in the presence of GenTegra™ at 25°C were nearly identical to those of control samples stored at -80°C, and the samples exhibited a median RIN that was 1.0 units higher than samples stored without protection. Remarkably, even samples stored at temperatures as high as 56°C were significantly stabilized by GenTegra™ compared to samples stored without protection.

Samples stored dry for 2 weeks at 56°C in the presence of GenTegra™ and control samples stored at -80°C were analyzed using Affymetrix Human Exon 1.0 ST Arrays. Samples stored at 56°C without protection were not suitable for analysis due to extensive degradation. To examine whether GenTegra™ inhibits the NuGEN labeling reaction or probing of the Affymetrix arrays, the percentage of probe sets called present by Affymetrix Expression Console software were compared. There was no difference in the percentage of present calls, with 59% called present when stored with GenTegra™ and 60% called present when stored at -80°C in the absence of GenTegra™ ( $p = 0.31$ ). Additionally, the

average expression level of probe sets called present was not different (7.9 vs. 7.8;  $p = 0.31$ ). Interestingly, there was no difference in background between the samples stored with or without GenTegra™, although the average background trended lower in the samples with GenTegra™ (115 vs. 129 respectively;  $p = 0.075$ ). Next, the correlation of raw expression values between arrays was examined (Table 2). The average of correlations between samples stored at 56°C (samples stored at 56°C compared to other samples stored at 56°C; for example, see Table 2, column 4, row 5) is not significantly different from those stored at -80°C (correlations of samples stored at -80°C compared to other samples stored at -80°C; for example, see Table 2, column 5, row 6). To determine whether the presence of GenTegra™ in the labeling reaction was inhibitory, the correlations among the 56°C samples and the -80°C samples (for example, see Table 2, column 4, row 4) were compared to those between the 56°C samples and the -80°C samples (for example, see Table 2, column 5, row 3). None of the groups of correlations showed differences, indicating that the presence of GenTegra™ RNA did not inhibit the labeling of samples or the probing of the arrays. The complete results can be viewed at [www.genvault.com/RNAworld.pdf](http://www.genvault.com/RNAworld.pdf)

## Conclusions

In the present study, we examined the use of GenTegra™ for storage of total RNA purified from PBMCs. Here, we have shown that the integrity of RNA stored in the liquid and dry states at various temperatures is preserved in the presence of GenTegra™, while samples stored without protection exhibit substantial degradation. We then compared the results of Affymetrix exon assays using RNA stored for two weeks at 56°C in the presence of GenTegra™ and control RNA stored at -80°C. No differences were observed between samples stored in GenTegra™ and frozen controls, indicating that GenTegra™ did not inhibit NuGEN labeling or probing of the arrays. In conclusion, GenTegra™ enables storage and transport of purified RNA at ambient temperature and is compatible with Affymetrix Human Exon 1.0 ST arrays. Thus, GenTegra™ assures preservation of valuable RNA samples without interfering with downstream analyses.

## References

1. Barnes MG, et al. *Arthritis Rheum* 2009;60(7):2102-12.
2. Barnes MG, et al. *Rheumatology (Oxford)* 2004;43(8):973-9.
3. Fall N, et al. *Arthritis Rheum* 2007;56(11):3793-804.
4. Griffin TA, et al. *Arthritis Rheum* 2009;60(7):2113-23.
5. Irizarry RA, et al. *Nucleic Acids Res* 2003;31(4):e15.

Sponsored Paper. *BioTechniques* 48:468-469 (June 2010)  
doi 10.2144/000113447