Improved RNA stability and integrity for better RNAseq data.

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Introduction

RNA-Seq is one of the most precise techniques available for transcriptomics analysis. This valuable tool is used across a wide range of life science applications such as cancer research, precision medicine and clinical trials. However, since this technique requires the analysis of one of the most labile biomolecules, good quality of the input RNA is of utmost importance.

Since RNA is susceptible to degradation even after purification, maintaining high quality RNA requires cold chain handling along the entire workflow from purification to processing, and/or shipping and storage. The use of Active Chemical Protection™ (ACP) is a viable and convenient alternative to ice for handling samples, or dry ice and freezers for shipping and storage.

ACP opens the possibility of collecting, handling, or shipping and storing samples without the need for cold chain, thereby facilitating the work with RNA at all points in the workflow. In this poster we show data related to GenTegra® products designed to protect RNA integrity at different stages of the sample workflow and that are completely compatible with techniques used in the laboratory and with downstream analysis such as RNA-Seq.

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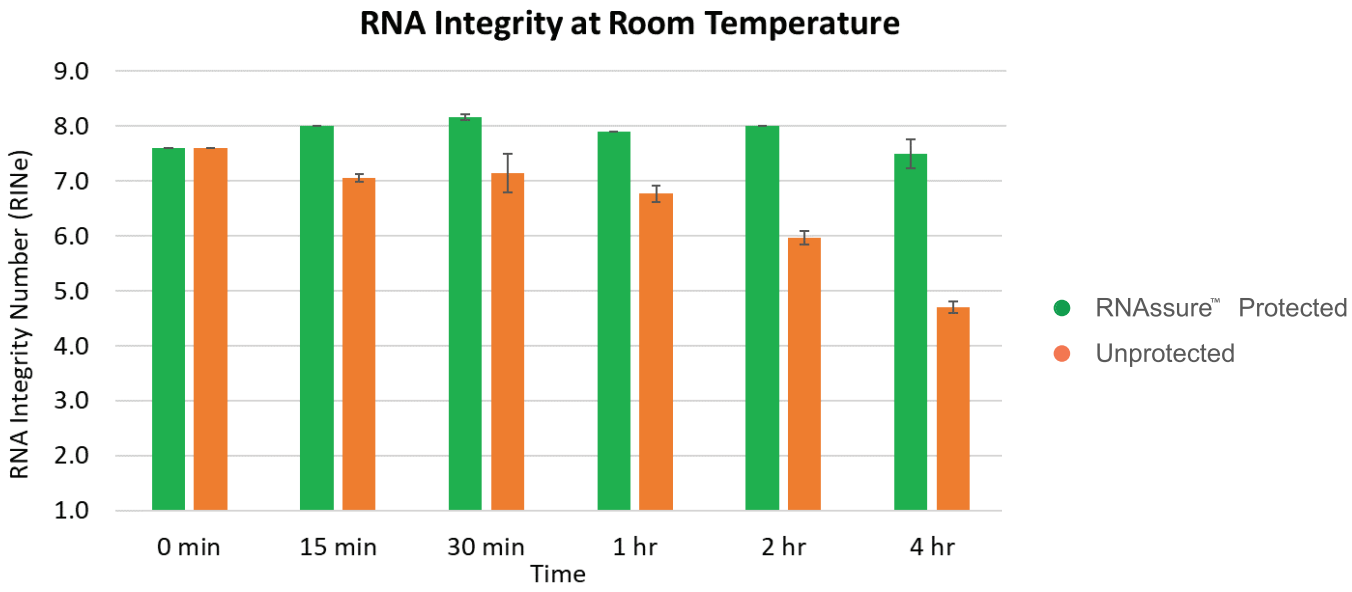
GenTegra RNAssure™; protects RNA samples at the bench at room temperature.

RNAssure™ short-term sample protection of RNA integrity



- RNA degradation occurs in a matter of minutes.
- Unprotected samples starting with a RINe of 7.5 quickly decay to RINe values of less than 6 in two hours and lower than 5 by four hours.
- RNAssure™ protected samples maintain RINe values of around 7.5 or higher at room temperature during the whole time course.

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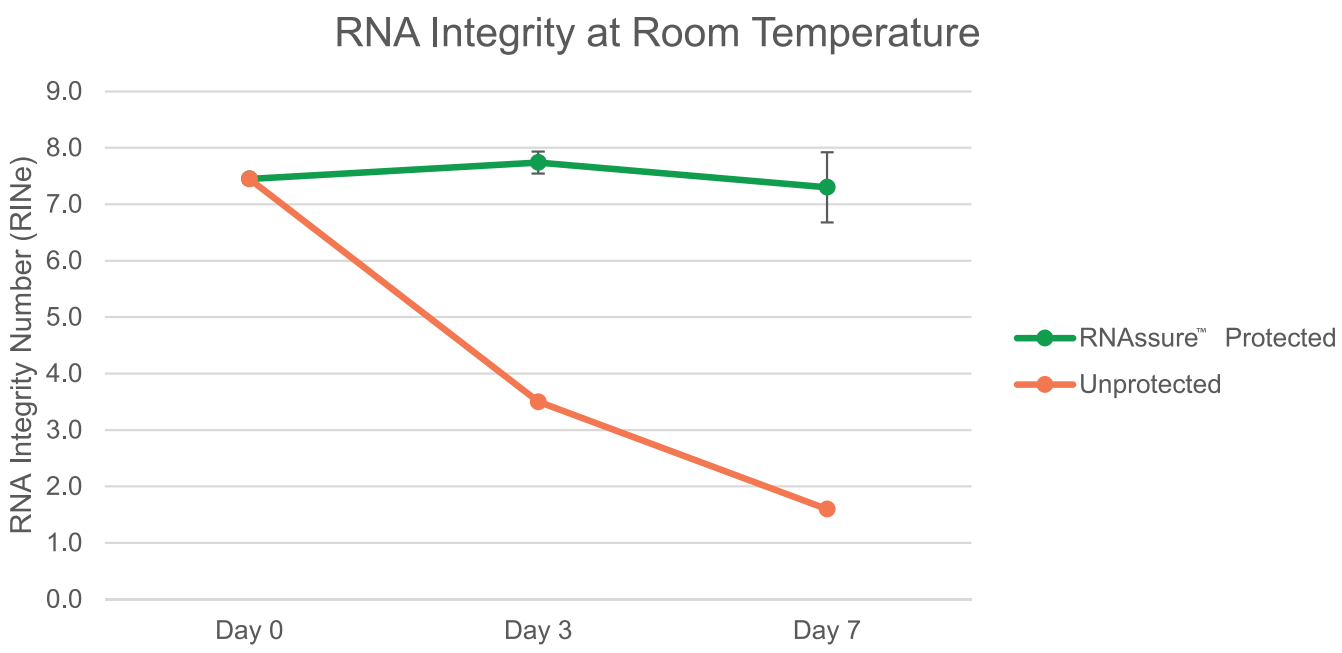

RNAssure mid-term protection of RNA integrity

- 20 to 50µL of 20 ng/µL mouse spleen RNA added to tubes protected with RNAssure or unprotected and left at room temperature (~25°C) for 7 days.
- Agilent Tape Station was used to analyze the RNA Integrity Number (RINe) of the samples at days 0, 3 and 7.
- While the RINe value dramatically decreases for unprotected samples until they are severely degraded (RINe < 3.0), RNAssure protected samples preserve a RINe > 7.0 for the duration of the incubation.

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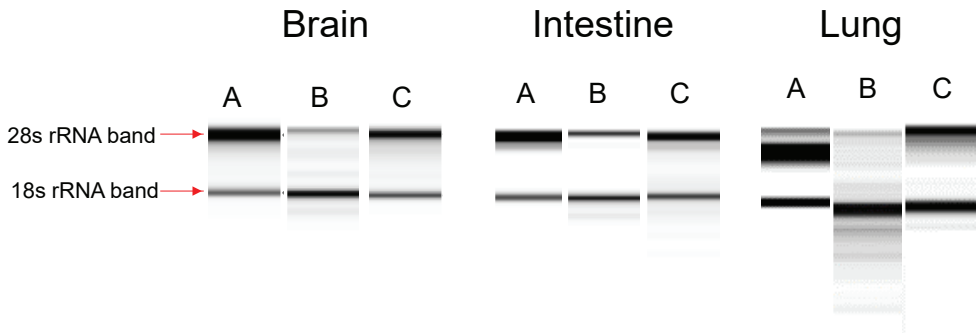
DNase treatment in presence of RNAssure™



A) Original Sample
B) Turbo DNase + No Protective Agent
C) Turbo DNase + RNAssure™

- For some DNase digestion protocols such asTurbo™ DNase, incubation steps at elevated temperatures are required: 30 minutes at 37°C for digestion and 10 minutes at 75°C.
- RNA extractions from different organs were submitted to DNase treatment in presence and absence of RNAssure™.
- The presence of RNAssure™ during in-solution DNase digestion helps maintain RNA integrity of the samples after the elevated incubation temperatures required for the process.

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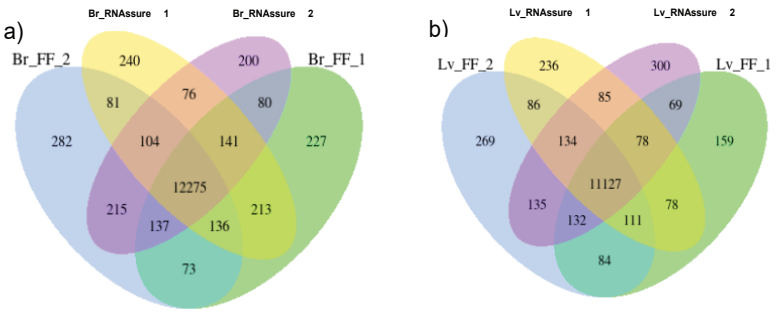
RNAssure™ doesn't have a detrimental effect on RNA-Seq



a) Venn diagram for uniquely expressed genes in RNA samples extracted from mouse brain samples

b) Venn diagram for uniquely expressed genes in RNA samples extracted from mouse liver samples

- The co-expression Venn diagram presents the number of genes that are uniquely expressed within each group/sample.
- The overlapping regions show the number of genes that are co-expressed in two or more groups/samples.
- The difference between any pair of samples is similar, including the two replicate/identical fresh frozen samples.
- This suggests that there is no significant impact of RNAssure on RNAseq results that is above natural variation.

Extracted tissue samples were frozen in tubes with no RNAssure™ , RNAssure samples were aliquots of the frozen samples kept in tubes containing RNAssure.

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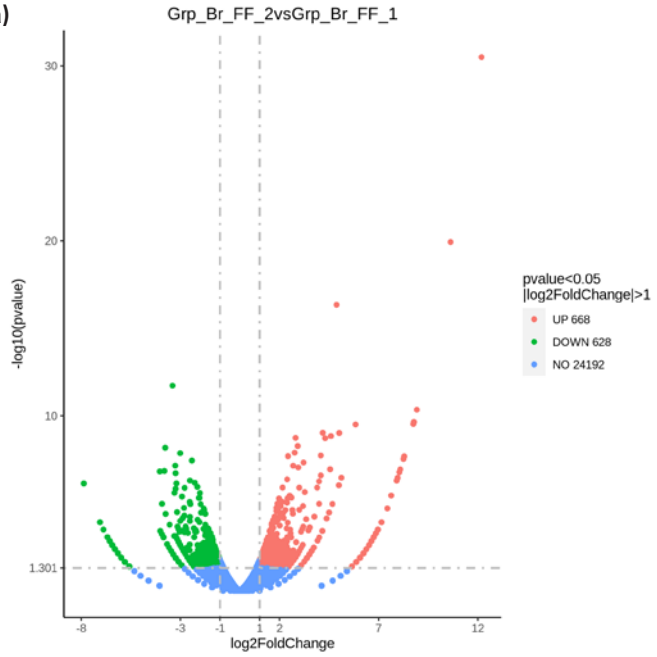
Gene expression differences between frozen tissue samples and samples with RNAssure™

a)



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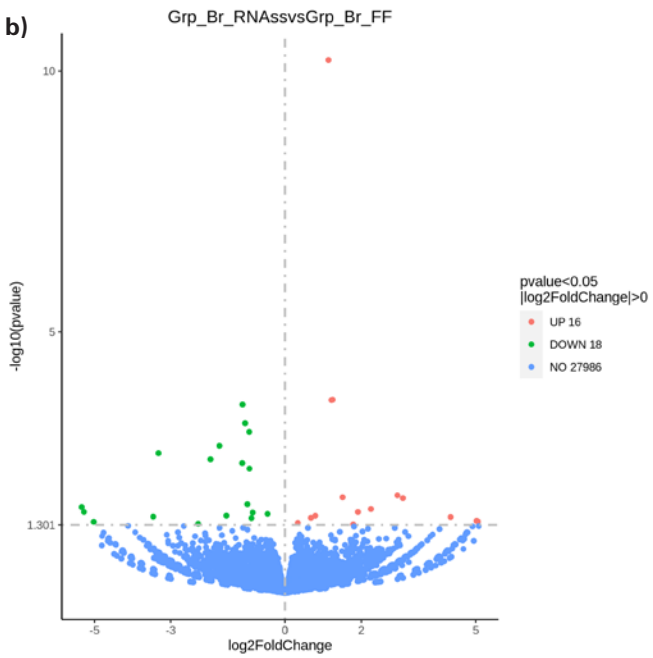

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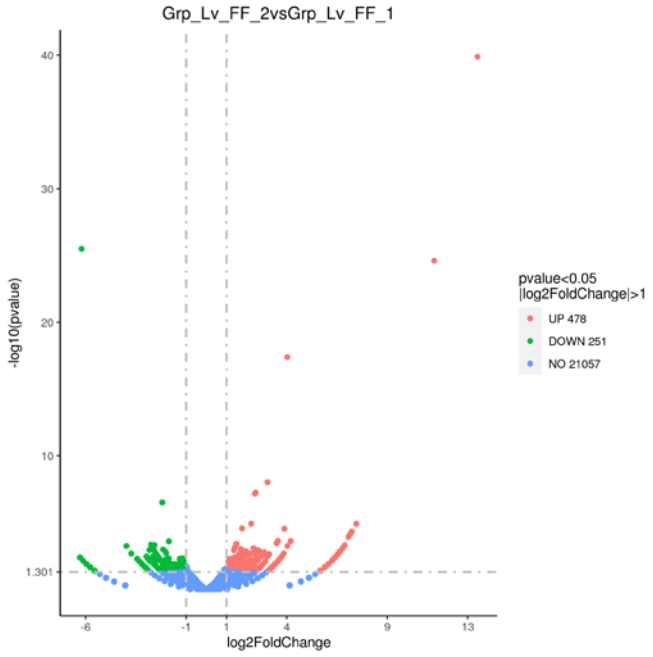

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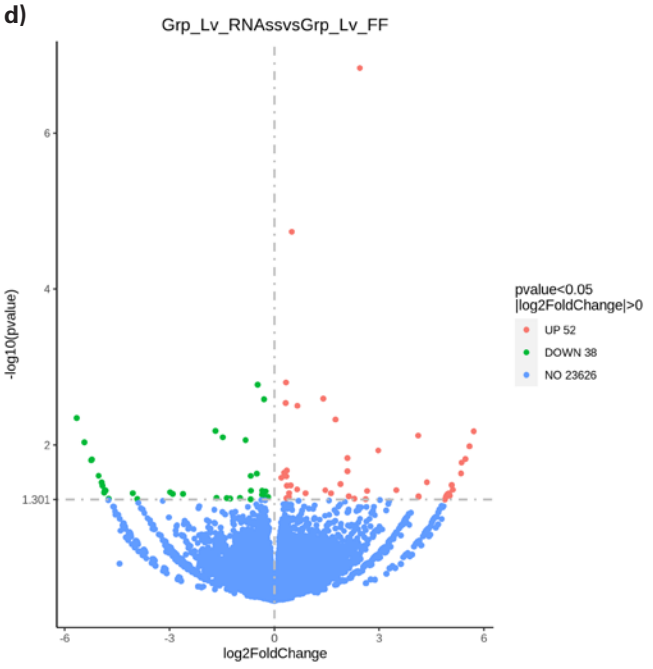

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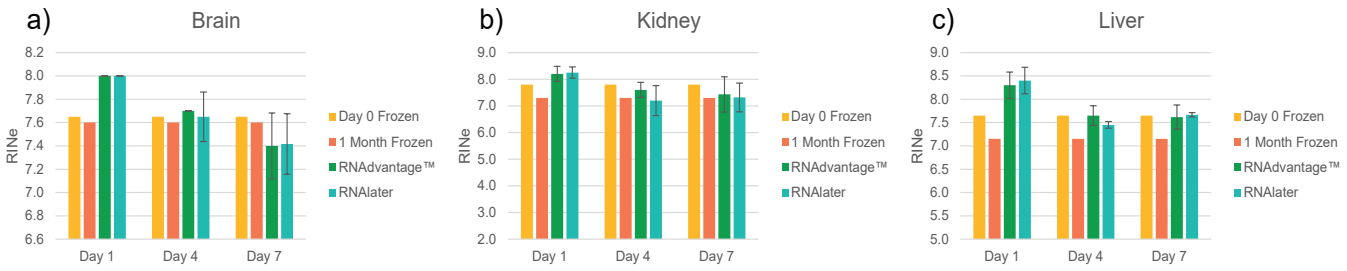
Volcano plots of differentially expressed genes between a) Brain frozen samples b) Brain frozen samples and RNAssure samples c) Liver frozen samples d) Liver frozen samples and RNAssure samples.

- The differentially expressed genes between pairs of frozen samples is between 3 to 5%.
- The differentially expresses genes between frozen samples and the same samples kept in RNAssure™ is lower than 0.5%.

GenTegra RNAdvantage™; stabilizes RNA in fresh tissue samples without freezing.

RNAdvantage™ preserves RNA integrity equivalent to frozen samples



- RINe scores from different mouse tissue samples kept frozen for 0 days and 1 month or kept at room temperature for up to 7 days in RNAlater™ and RNAdvantage™.

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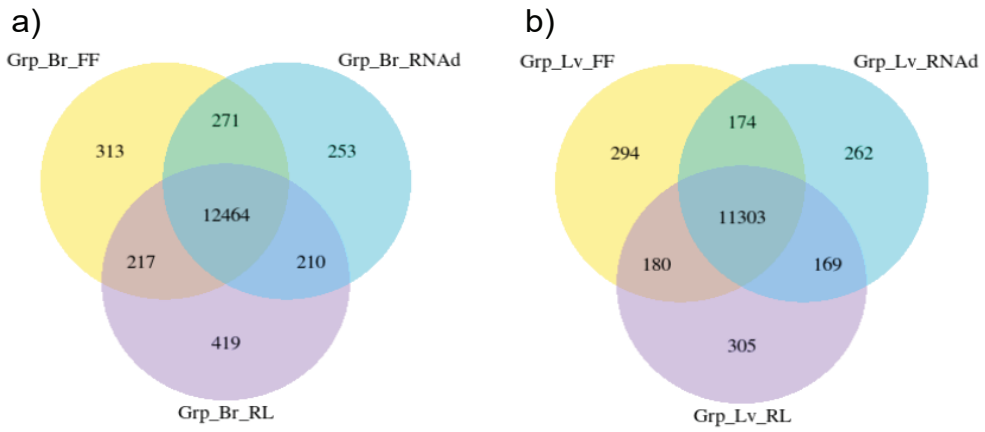
- In all cases RNAdvantage™ demonstrates protection of RNA integrity, and in most cases protection is equivalent or superior to 1 month storage at -80°C.

RNAdvantage™ has no detrimental impact on RNA-Seq



- Venn diagrams for a) Brain and b) Liver samples comparing fresh frozen samples with samples stored in RNAdvantage or RNAlater for 7 days at RT.
- The co-expression Venn diagram presents the number of genes that are uniquely expressed within each group/sample with the overlapping regions showing the number of genes that are co-expressed in two or more groups/samples.
- The difference between any group of samples regardless of storage condition is similar.
- This suggests that there is no significant impact of RNAdvantage or RNAlater on RNAseq results that's above natural variation.

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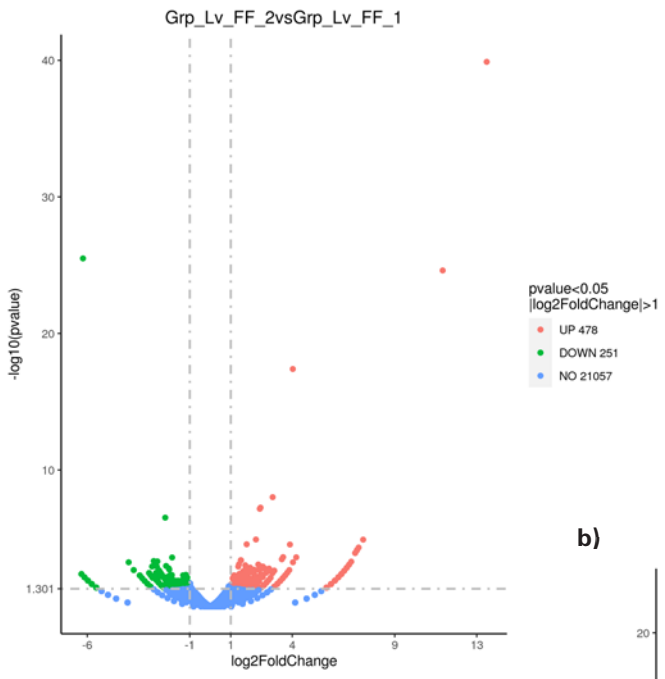
Gene expression differences between frozen liver samples and with RNAdvantage™

- Comparing both frozen samples between themselves and to samples in RNAdvantage™ reveals a similar percentage of differentially expressed genes.
- RNAdvantage™ doesn't have a significant impact on sequencing results beyond natural variation between samples.

a)



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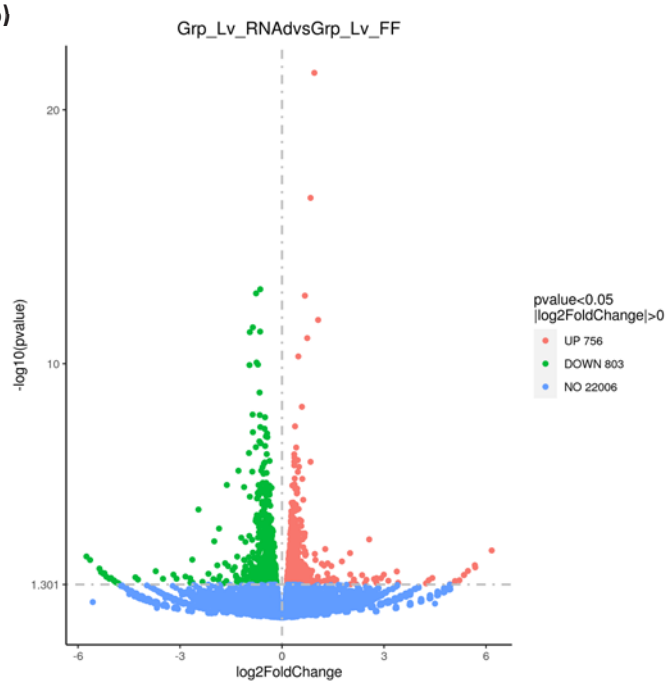

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Summary

We present data that proves the protective effect of RNAssure™ and RNAdvantage™ in preserving RNA quality in samples before and after purification.

RNAssure™ results of short-term and mid-term protection at room temperature for liquid RNA samples are shown, as well as its potential protective use in other sample treatment techniques such as DNase digestion and RNA sequencing.

RNAdvantage™ data shows the protective effect on RNA from fresh tissue compared to a similar product and with freezing the samples, as well as data showing that it doesn't interfere with RNA sequencing.

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