Application Note

High quality RNA from heat stabilized samples

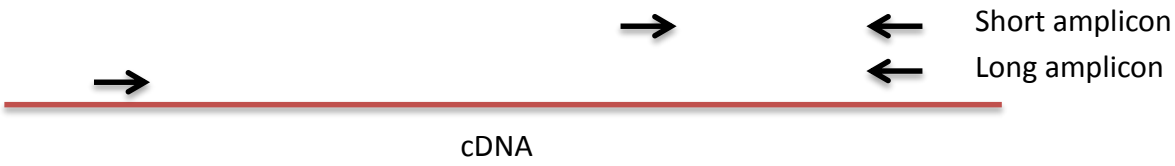
Introduction

The ability to analyse both RNA and protein from a single sample are required in some studies. This can be problematic since the two analytes have different requirements in order to be preserved in a high quality state. Standard RNA preservation strategies, e.g. RNA later, while preserving RNA quality do not prevent the more rapid changes to proteins and their modifications. Heat induced enzymatic inactivation have been shown to be an efficient way to preserve proteins and their modifications but RNA quality have been a concern in such samples. RNA quality as measured using electrophoretic analysis of rRNA, e.g. Bioanalyzer assay, has indicated a reduction in quality due to heating. In the work presented here it is shown that RNA quality when based on mRNA integrity as measured with qPCR is not effected by heating. Although heat inactivation does not stabilize RNA it enables a combination protocol where proteins and their modifications can be preserved by heat stabilization and in a second step mRNA can be preserved using standard RNA preservation strategies, *e.g.* rapid freezing or RNA Later.

Results

mRNA quality measured using qPCR measure the difference in Ct between two qPCR reactions, a long and a short amplicon, from the same transcript, see schematic in fig. 1. This is a more direct and meaningful measure compared to the traditional methods based on rRNA which only indirectly measures RNA quality (1).

Fig. 1 Schematic of principle for qPCR based quality measurement of RNA



qPCR has been used to measure mRNA quality with three different qPCR based assays based on three different mRNAs reflecting different modes of degradation:

- 18S assay for assessment of physical degradation.
- Degradation assay for assessment of enzymatic degradation.
- Stability assay for general mRNA stability.

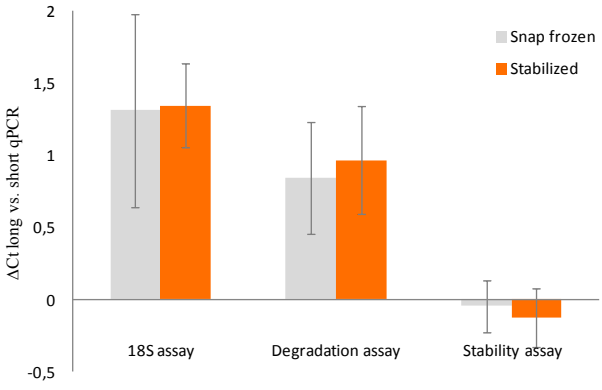


Fig.2 ΔCt for 3 qPCR RNA quality assays

All three qPCR based assays show comparable mRNA quality between heat stabilized samples and traditionally snap frozen samples, fig. 2.
RNA quality as measured using electrophoretic analysis of rRNA, *e.g.* Bioanalyzer assay, shows a decrease of about 1 unit for the RIN value, fig 3. This indicates a preferential heat induced degradation of rRNA, as measured by the RIN value, whereas the more robust mRNA is not similarly affected by the short heating in the Stabilizor™ system, as evident by the ΔCt measurements.

application@denator.com/ www.denator.com
Jan 2016

Material and Methods

Rat brain tissue from adult Wistar rats was harvested directly after CO2 sacrifice. Two adjacent sagital sections of 1 mm thickness were cut at the Y-point. One section was heat stabilized using the Stabilizor system in Auto Fresh mode and then flash frozen in liquid nitrogen while the other section was directly snap frozen, fig. 4. Extraction and subsequent RNA analysis was performed at TATAA Biosciences, Sweden, an ISO 17025 certified contract lab (2). Briefly the samples were processed as follows: The samples were homogenized while frozen and extraction buffer added directly to the frozen powder. RNA was extracted using QiaZol and Qiagen RNeasy Mini Kit. RIN-values were measured on BioAnalyzer (Agilent Technologies) and cDNA was synthesized using TATAA Grandscript cDNA synthesis kit #A103 (TATAA Biocenter AB). qPCR was performed with TATAA SYBR® GrandMaster Mix # TA01 (TATAA Biocenter AB) and all samples were run in duplicates on LightCycler480 platform (Roche, Inc).

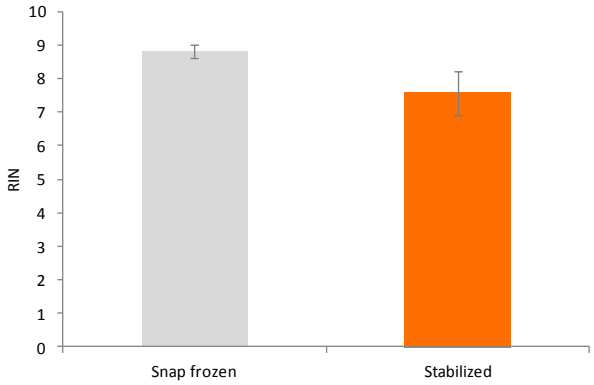


Fig. 3 RIN value for snap frozen and heat stabilized brain samples

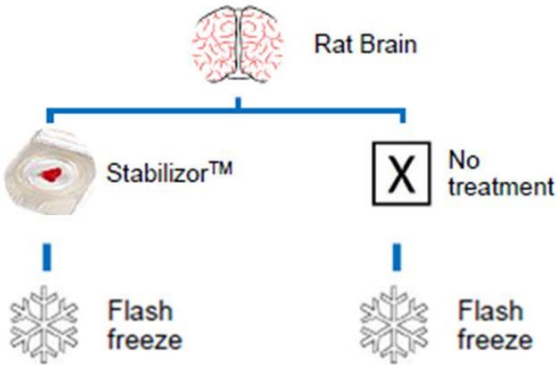


Fig. 4 Layout of experiment

References

- Patent WO2013139860: METHODS FOR ASSESSING RNA QUALITY http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=WO&NR=2013139860A1&KC=A1&FT=D&ND=4&date=20130926&DB=worldwide.espacenet.com&locale=en_EP

- J. Björkman, et at., Differential Amplicons (ΔAmp) - a new molecular method to assess RNA integrity. Biomol. Detect. Quantif. (2015)