



APPLICATION NOTE

Novel Dry Storage Approach for RNA Preservation at Room Temperature

► Introduction

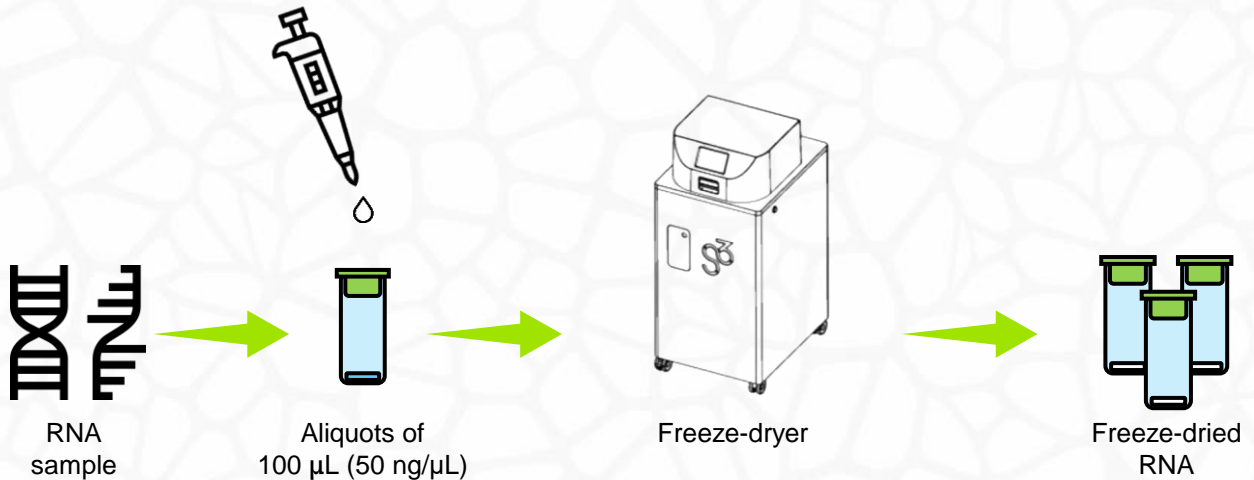
Researchers need access to high quality biological samples to be used both for clinical and research purposes. Because of that, a wide range of primary samples and their subproducts may be processed and stored in either short or long-term. The most extended method for long-term preservation of RNA is ultra-low temperature (ULT) freezing at $-80\text{ }^{\circ}\text{C}$. However, maintaining those protective conditions implies high maintenance costs, large spaces, constant energy supply, transport constrains and safety measures to minimize the risk of losing collections.

Here, *300K Solutions* propose an alternative approach for room temperature (RT) preservation based on RNA freeze-drying and stabilization that has the potential to be a real alternative to the current ULT RNA storage.

► Materials and methods

RNA was obtained from the peripheral blood of one donor using the organic solvent method. Once extracted, RNA aliquots of $100\text{ }\mu\text{L}$ ($50\text{ ng}/\mu\text{L}$) were stored under two different conditions: frozen at $-80\text{ }^{\circ}\text{C}$ (control) and dried at RT ($22\text{ }^{\circ}\text{C}$).

Workflow

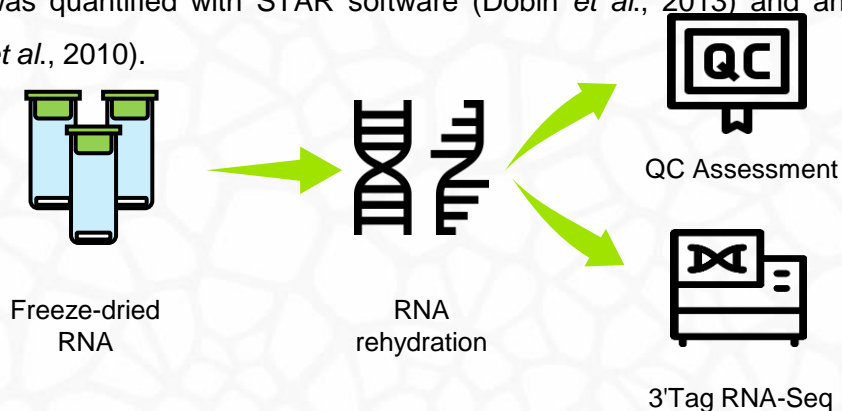


Then, after 1, 3 and 8 weeks one aliquot of each condition was recovered by either thawing (samples maintained at -80 °C) or rehydrating with molecular biology grade water (samples maintained at RT). Each aliquot material was then subjected to a basic quality control (QC) procedure following the Proficiency Standards established by the *International Society for Biological and Environmental Repositories (ISBER)*:

- RNA quantity and purity by spectrophotometry.
- RNA quantity by fluorometry.
- Determination of RNA integrity by agarose gel electrophoresis.
- RNA integrity analysis by Agilent 2200 TapeStation System.

Finally, to assess the suitability of the freeze-dried samples for genomic studies and notice if there were differences between both storage conditions through time, we performed *3' Tag RNA-Seq* assay.

Gene expression was quantified with STAR software (Dobin *et al.*, 2013) and analysed with EdgeR (Robinson *et al.*, 2010).



► Results

QC assessment was performed in RNA samples stored at RT (22 °C) or -80 °C for up to 2 months. This quality control focused on RNA integrity (RIN) (Table 1). Once we confirmed that our stabilization solution preserve high quality RNA (RIN>7), we decided to assess its functionality using 3'Tag RNA-Seq assay.

Sample ID	Time of storage	Way of storage	RIN
RNA T ₁	1 week	-80 °C	7.90
		RT	7.80
RNA T ₂	3 weeks	-80 °C	7.90
		RT	8.10
RNA T ₃	8 weeks	-80 °C	8.20
		RT	8.30

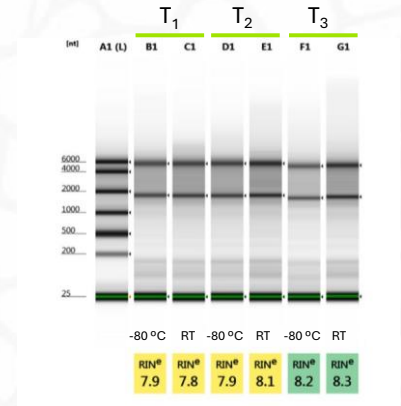


Table 1. RNA integrity in frozen or freeze-dried RNA samples after 1, 3 or 8 weeks of storage.

The results of this analysis showed no significant differences in the number of reads and the gene expression in both samples (stored at -80 °C or RT) through time (Table 2 and Figure 1).

Sample ID	Time of storage	Way of storage	% (Aligned readings)	Number of readings
RNA T ₁	1 week	-80 °C	82.3%	1.6M
		RT	81.9%	1.7M
RNA T ₂	3 weeks	-80 °C	84.6%	1.3M
		RT	82.3%	1.2M
RNA T ₃	8 weeks	-80 °C	84.0%	1.5M
		RT	81.2%	1.5M

Table 2. Gene expression quantifying in frozen or freeze-dried RNA samples after 1, 3 or 8 weeks of storage.

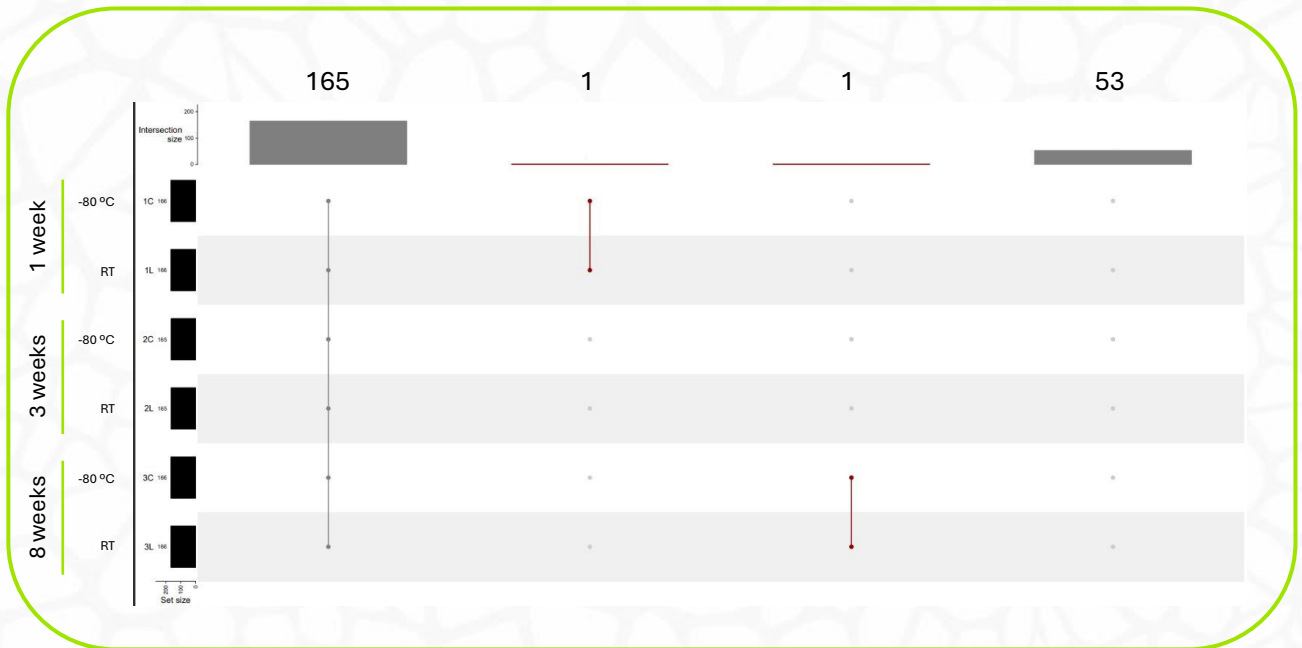


Figure 1. UPSET diagram showing the expressed genes shared among the different samples. Expression criteria: to have more than 500 aligned readings.

► Conclusions

1. 300K Solutions has developed a methodology that aims to offer a standardized stabilization procedure for biological materials that allows their storage at RT.
2. The technology here proposed offers protection during the freeze-drying process of RNA resulting in stability at RT and potentially becoming an alternative to ULT storage. This could allow the use of dried RNA samples for shipping.
3. The methodology developed by 300K Solutions aims to offer the use of freeze-dried RNA samples in downstream applications such as RNA-Seq.

► References

Images: Flaticon