DNA Preservation and Storage at Room Temperature for Shipping

Introduction

DNA storage under optimal conditions has become critical to ensure the quality of this genetic material, enabling its use in both research and clinical studies[1,2,3]. In addition to long-term storage, it is also crucial to maintain DNA integrity and functionality during transport between facilities. Unlike other types of biological samples such as tissues, cells and RNA, and due to its nature, DNA can be shipped with less restrictive conditions (room temperature). However, to ensure the suitability of DNA samples for complex Molecular Biology techniques like NGS studies it is recommended to ship DNA under refrigerated conditions which results in higher costs and safety risks.

Here, we propose an alternative approach for DNA shipping at room temperature (RT) based on DNA drying and stabilization, designed to fit research facilities' workflow. This technology, which we have named DNA Shipping Solution, is based on the use of tubes coated with a stabilization buffer that protects DNA against RT associated degradation and has the potential to be a real alternative to the current DNA shipping standards. Simply add purified DNA, mix gently and dry, and your sample is ready to be shipped. Upon arrival and when needed, DNA can be rehydrated using molecular biology grade water.

This white paper presents preliminary data on the quality, integrity and functionality of DNA stabilized, dried, and stored for 1 month at RT, under protective and unprotective conditions (with or without the presence of a stabilization buffer), comparing them with those of DNA stored under "control conditions" (frozen at -20 ºC).

Study design

Four peripheral blood samples were obtained from healthy donors. From each one of these samples, DNA was extracted by Salting Out (protocol available at the Spanish Biobank Network SOPs: https://brd.nci.nih.gov/brd/sop/show/1070). Once extracted, 3 DNA replicates from each donor were stored under three different conditions: frozen at -20 ºC to be used as control, and dried at RT (22 ºC) with or without the presence of a stabilization buffer. Finally, after 1 month, the three replicates from each condition

Authors

Ana Hernández[1], Miguel G. Álvarez[1], Marta Martín-Ayuso[1]

[1]300K Solutions, Salamanca, Spain

were recovered and either thawed (liquid samples stored at -20 ºC) or rehydrated with molecular biology grade water to return the DNA to its initial concentration (dried samples stored at 22 ºC with or without stabilization buffer). Each aliquot material was then subjected to a basic quality control (QC) procedure according to the standards of the Banco Nacional de ADN (BNADN) (https://www.bancoadn.org/docs/programa-control-calidad-muestras.pdf) and compared with aliquots in which QC assessments were performed right after DNA extraction (fresh DNA). An overview of the experiment plan can be visualised in Figure 1.

QC assessment included the following procedures that provide objective information on sample concentration, purity, integrity and functionality:

- DNA purity by spectrophotometry.
- Determination of DNA integrity by agarose gel electrophoresis.
- Determination of double-strand DNA concentration by fluorimetry.
- DNA integrity analysis by Agilent 2200 TapeStation System from Agilent Technologies.
- DNA functionality and integrity by multiplex long PCR.

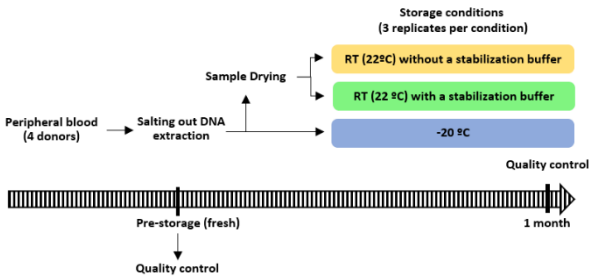

Figure 1. Flowchart summarizing the study design. Genomic DNA was obtained from peripheral blood of four donors using the Salting Out extraction method. Three DNA replicates form each one of these 4 donors was aliquoted and either dried or frozen and stored at -20ºC. Dried aliquots were stored at 22 ºC in the presence or absence of a stabilization buffer. DNA samples obtained from each donor were submitted to a QC assessment just after extraction to obtain a baseline evaluation (fresh DNA). The QC assessment was then repeated after 1 month, using the aliquots stored under the different conditions (-20 ºC and 22ºC with or without buffer).

Results

DNA Shipping stabilization buffer maintain DNA purity, integrity and functionality.

Absorbance ratios 260/280 were measured in the different samples to assess DNA purity. As shown in Table 1, minimum variations were observed in the 260/280 ratios of all the samples analysed ("fresh" DNA and DNA stored for 1 month under three different conditions: frozen at -20ºC and stored at RT with and without the presence of a stabilization buffer). Moreover, for 260/230 ratios the values throughout storage time went from 2,31 ± 0.004 in fresh to 1,86 ± 0.06 after 1 month at RT in the presence of a stabilization buffer*. All in all, all values are within the acceptance criteria stablished by the BNADN.

Table 1. Measurement of DNA purity and integrity with standard methods. Table comparing the median values ± SD of A260/280 and A260/230 ratios and DNA integrity number (DIN) of fresh DNA and DNA stored for 1 month under three different conditions (frozen at -20ºC and stored at RT with and without the presence of a stabilization buffer).

	A260/280 ratio	A260/230 ratio	DIN
Fresh DNA	1,90 ± 0,01	2,31 ± 0,03	8,85 ± 0,06
1 month (-20 ºC)	1,87 ± 0,01	2,08 ± 0,01	8,93 ± 0,21
1 month (22ºC without buffer)	1,88 ± 0,02	2,07 ± 0,04	9 ± 0,18
1 month (22ºC with buffer)	1,88 ± 0,02	1,86 ± 0,06*	8,93 ± 0,20

*Due to the nature of the components of the stabilization buffer, when assessing DNA concentration and purity using a Nanodrop, an underestimation of the 230/260 absorbance ratio between ≈ 0.14 – 0.24 is observed.

In addition, all samples under study didn't show significant signs of degradation as assessed by agarose gel electrophoresis (Figure 2) and therefore, their integrity was further assessed using the Agilent 2200 TapeStation System. DIN (DNA integrity number) values from all the samples were higher than 8,5 which according to the BNADN standards indicate an excellent DNA integrity (Table 1).

After optimum DIN values were obtained, we performed a multiplex long PCR to assess DNA functionality. Agarose gel score using the PCR products reported a 17.5 kb band which was clearly distinguishable in DNA stored for a 1 month frozen at -20ºC and at RT in the presence of stabilization buffer. However, in the case of the samples stored for 1 month at RT without the presence of the stabilization buffer this 17.5 Kb was weaker or even undetectable, suggesting a loss of DNA functionality and integrity (Figure 3).

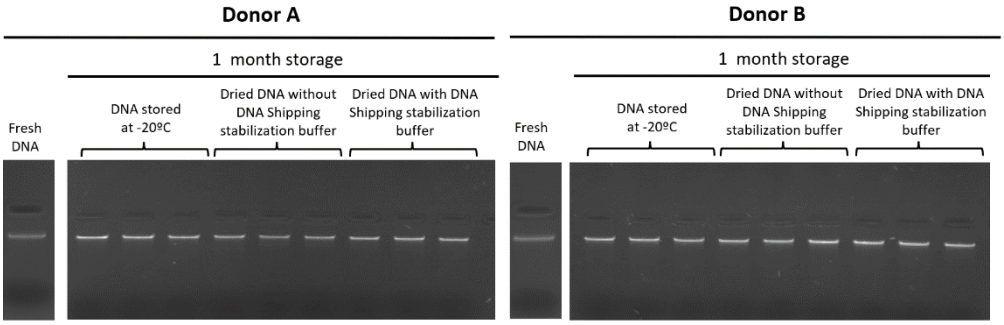

Figure 2. DNA integrity assessed by agarose gel electrophoresis. DNA from 4 donors was loaded in a 0,8% agarose gel electrophoresis to tests its integrity during the storage time (1 month under three different conditions: frozen at -20ºC and stored at RT with and without the presence of a stabilization buffer). The results of the fresh DNA are also shown. None of the samples show significant signs of degradation. The same results were observed for donors C and D.

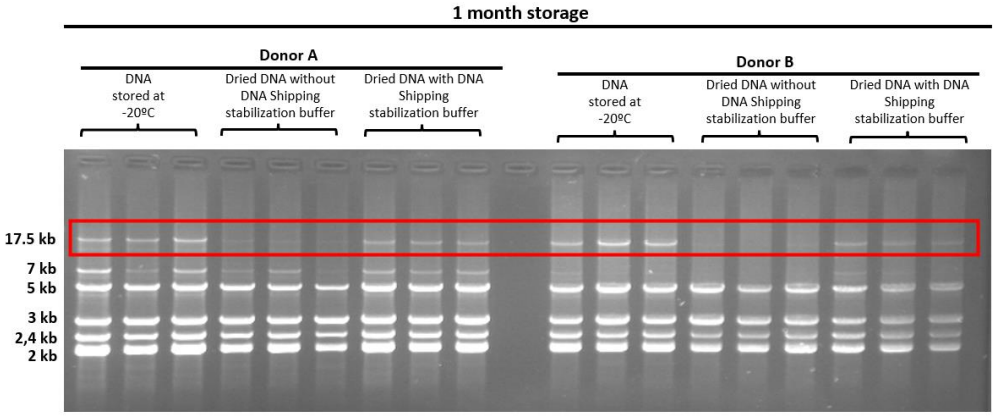

Figure 3. DNA Shipping stabilization buffer maintains the functionality and integrity of DNA dried and stored at RT. To assess the integrity and functionality of the DNA dried using the DNA shipping tubes we performed a multiplex long PCR with DNA stored for 1 month using three different conditions: frozen at -20ºC (reference condition) and dried at RT with or without the DNA shipping stabilization buffer. As shown above, a defined band of 17.5 Kb (red rectangle) is observed in both DNA stored at -20ºC and at RT with the DNART shipping stabilization buffer, whereas the DNA stored at RT without this buffer showed an almost undetectable 17.5 Kb band, suggesting a loss in DNA functionality and integrity. The same results were observed for donors C and D.

DNA Shipping stabilization solution protects against DNA loss after RT storage.

To assess the % of DNA loss after RT storage, we measured double-strand DNA concentration using a Qubit Flex of all the DNA samples stored for 1 month under the three different conditions (frozen at -20 ºC and dried at RT with or without a stabilization buffer) and compared it with the concentration of fresh DNA.

As shown in Figure 4, the DNA samples stored for 1 month at RT without a stabilization buffer showed a higher DNA loss when compared with DNA stored for 1 month at RT in the presence of the stabilization buffer. More importantly, there was no difference in terms of DNA loss between DNA with stabilization buffer and DNA stored under control conditions (frozen at -20 ºC), supporting the suitability of the DNART stabilization buffer for DNA shipping at RT temperature.

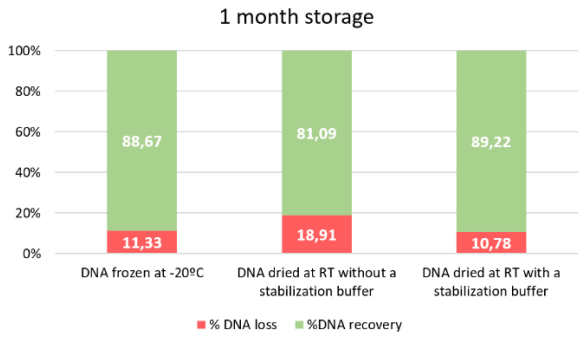

Figure 4. DNA Shipping stabilization buffer protects against DNA loss after RT storage. The quantification of double-strand DNA concentration showed that, when compared with fresh DNA, the presence of the DNA Shipping stabilization buffer has a protective effect against DNA loss during RT storage when compared with DNA dried and stored at RT without a stabilization buffer. Moreover, no difference in terms of DNA loss was observed between DNA stored for 1 month frozen at -20 ºC and DNA stored for 1 month at RT in the presence of a stabilization buffer.

Conclusions

300K Solutions has developed a methodology that aims to offer the possibility of shipping DNA samples dried at room temperature. Based on the results obtained in this work, it is clear that the technology here proposed could preserve the integrity of these samples for at least 1 month, enough for any type of shipping. In summary, our findings have demonstrated that:

- DNA Shipping stabilization buffer maintains DNA purity, integrity, and functionality.
- DNA Shipping stabilization buffer protects against DNA loss after RT storage.

References

1. Matange K, Tuck JM, Keung AJ. DNA stability: a central design consideration for DNA data storage systems. Nat Commun. 2021;12(1): 1358

2. Paskal W, Paskal AM, Dębski T et al. Aspects of Modern Biobank Activity – Comprehensive Review. Pathol. Oncol. Res. 2018;24(4): 771–785.

3. Coppola L, Cianfone A, Grimaldi AM et al. Biobanking in health care: evolution and future directions. J Transl Med. 2019; 17:172.

300K Solutions Parque Científico de la Universidad de Salamanca

Edificio M3, PB03, 37185. Calle Adaja 10, Villamayor. Salamanca (Spain)

admin@300K.bio www.300K.bio