White Paper

DNA Preservation and Storage at Room Temperature

Authors

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Introduction

Biobanks are technological platforms responsible for collecting, processing and storing biological samples, together with their associated data, to provide researchers access to high quality biological samples to be used both for clinical and research purposes. Depending on each biobank's nature and field, a wide range of primary samples and their subproducts may be processed and stored, employing different methodologies in the processing and storage of the biospecimens in either short or long-term[1]. In this context, the most extended method for long-term preservation of DNA is ultra-low temperature (ULT) freezing at -80 ºC[2,3]. However, maintaining those protective conditions implies high maintenance costs, large spaces, constant energy supply and safety measures to minimize the risk of missing collections[4,5]. In addition, sample transport at ULTs also results in high costs and safety risks.

Currently, there are several alternatives to the ULT freezing storage of DNA samples in the market which allow its storage under room temperature (RT) conditions (GenTegra; Biomatrica; Imagene). RT preservation approaches have the potential to avoid most of the ULT issues, having relatively modest upfront costs and requiring minimal space and basic overhead to maintain temperature.

Here, we propose an alternative approach for RT preservation based on DNA drying and stabilization, designed to fit biobank's workflow. Our storage solution, in essence similar to the one employed by the pharma industry for many drugs, has the potential to be a real alternative to the current ULT DNA storage.

This white paper presents preliminary data on the quality, integrity and applicability of DNA stabilized, dried and stored for different periods of time, under real (22 ºC) and simulated (60 ºC to recreate accelerating aging conditions) RT conditions, comparing them with those of DNA stored under "gold standard" conditions (frozen at -80 ºC).

Study design

Two different peripheral blood samples were obtained from healthy donors. From each blood sample, DNA was obtained using three different extraction methods: Salting out, Phenol/Chloroform (both protocols available at the Spanish Biobank Network SOPs: https://brd.nci.nih.gov/brd/sop/show/1070), and QIAamp DNA blood maxi kit (QIAGEN, Venlo, Netherland). Once extracted, DNA from each one of these 6 samples (2 donors and 3 extraction methods) was stored under three different conditions: frozen at -80 ºC to be used as control, and dried at RT (22 ºC) or 60 ºC (to evaluate the effects of storage under accelerating aging conditions based on the Arrhenius reaction rate function[6]). Finally, after 1, 6, 12 and 18 months (which corresponds to approximately 1, 6, 13 and 20 years at RT for the aliquots stored at 60 ºC, respectively) one aliquot of each condition was recovered and either thawed (liquid samples stored at -80 ºC) or rehydrated with Milli-Q H_2O to return the DNA to its initial concentration (dried samples stored at 22 ºC and 60 ºC). 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) before testing their suitability for other studies, and compared with aliquots in which QC and suitability 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 quantity and purity by spectrophotometry
- Determination of DNA integrity by agarose gel electrophoresis
- DNA integrity analysis by Agilent 2200 TapeStation System from Agilent Technologies
- Functionality and DNA integrity by multiplex long PCR

Finally, in order to assess the suitability of the dried samples for genomic studies we performed two of the most common genetic analysis:

- Whole Exome Sequencing (WES) performed and analysed by NIMGenetics (Madrid, Spain).

- Single nucleotide polymorphism (SNP) arrays using the Array CGH CytoScan® 750K (Affymetrix, ThermoFisher Scientific, Waltham, MA, US) performed by Unidad de Genómica del Instituto de Biología Molecular y Celular del Cáncer (IBMCC).

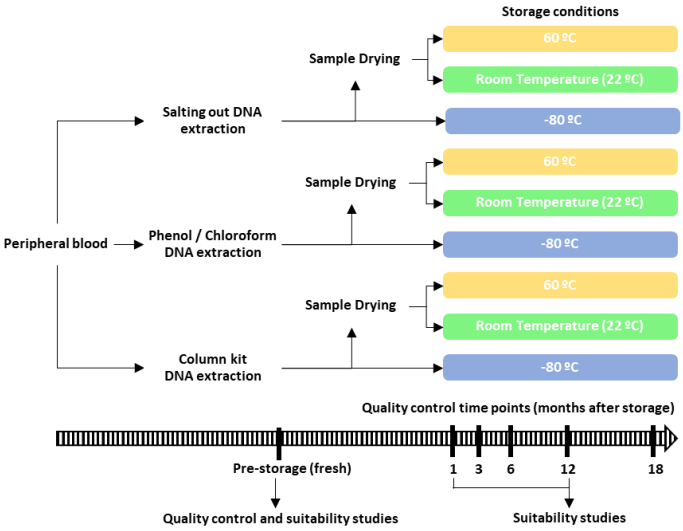

Figure 1. Flowchart summarizing the study design. Genomic DNA was obtained from peripheral blood of two donors using 3 different extraction methods in parallel - Salting Out, Phenol/Chloroform and QIAamp DNA blood maxi kit - resulting in a total of 6 different DNA samples. Each one of these 6 samples was aliquoted and either dried or frozen and stored at -80ºC. Dried aliquots were sealed under vacuum and either stored at 22 ºC or at 60 ºC (to generate accelerating aging conditions). Each sample obtained was submitted to a QC and suitability assessment just after extraction to obtain a baseline evaluation (fresh DNA). The QC assessment was then repeated at different time points (1, 6, 12 and 18 months), using the aliquots stored under the different conditions (-80 ºC, 22 ºC and 60 ºC) while the suitability assessment was repeated only after 1 and 12 months of storage.

Results

DNA purity, integrity and functionality. Absorbance ratios 260/280 were measured in the different samples that were purified with different extraction methods at all the time points to predict how long RT samples could be stored. As shown in Figure 2A, minimum variations were observed in the 260/280 ratios of all the samples, showing a median value ± standard deviation (SD) of 1.82 ± 0.02 in fresh and 1.80 ± 0.02 after 18 months at 60 ºC (Figure 2D). For 260/230 ratios the values throughout storage time went from 2.09 ± 0.14 in fresh to 1.97 ± 0.11 after 18 months at 60 ºC (Figure 2B and 2D).

In addition, the samples under study didn't show significant signs of degradation as assessed by agarose gel electrophoresis at the different time points (Figure 3) and therefore, their integrity was further assessed using the Agilent 2200 TapeStation System. DIN (DNA integrity number) values were measured exactly at the same time points in the same samples,

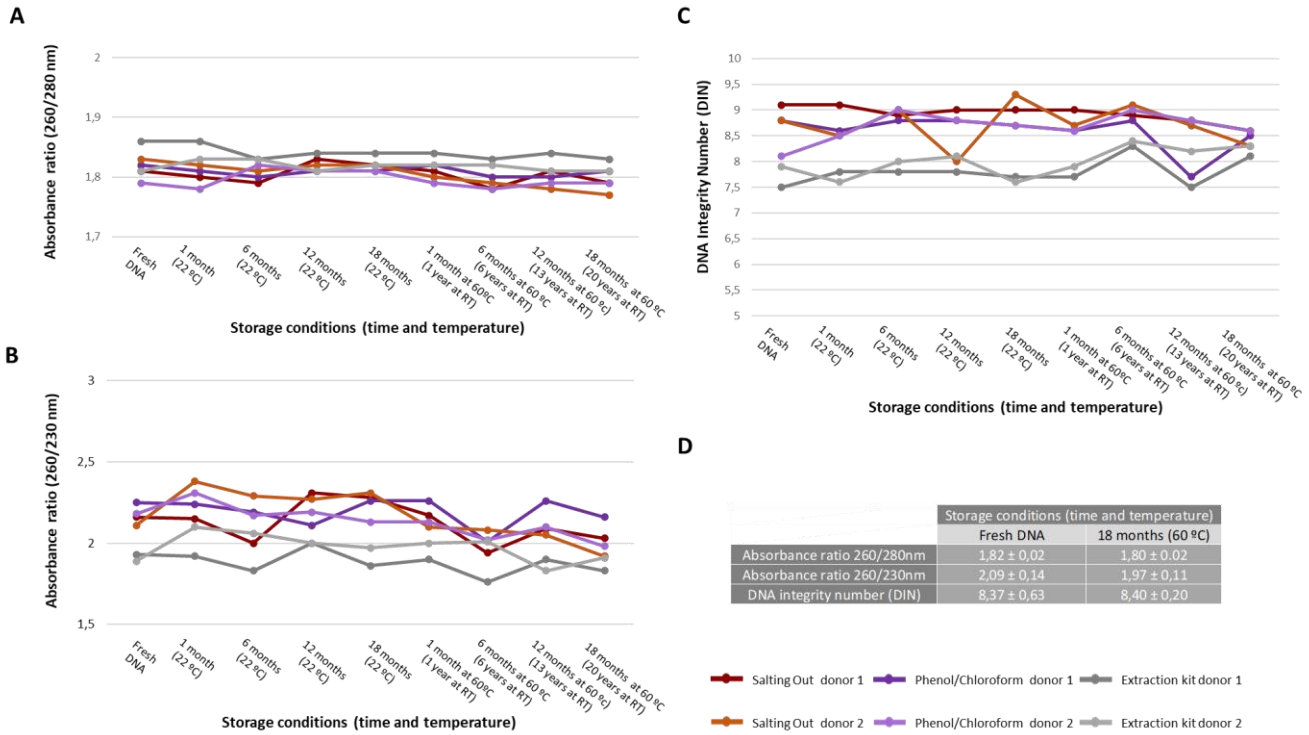

Figure 2. Measurement of DNA purity and integrity with standard methods. Evolution of several quality assessment measures during the storage time (1, 6, 12 and 18 months) for each individual sample (6 samples) under study. Time points are represented in terms of real storage time under different conditions (22 ºC and 60 ºC). Panels A and B show data on DNA purity as reflected by the light absorbance measurements (260/280nm and 260/230nm ratios respectively). Panel C shows data on the DNA Integrity Number (DIN) obtained from a 2200 TapeStation System. Panel D shows a table comparing the median value ± SD from all 6 samples studied in two different storage conditions (Fresh DNA and DNA dried and stored for 18 months at 60 ºC). For simulated RT conditions (60 ºC) storage for 1, 6, 12 and 18 months corresponds to approximately 1, 6, 13 and 20 years at RT.

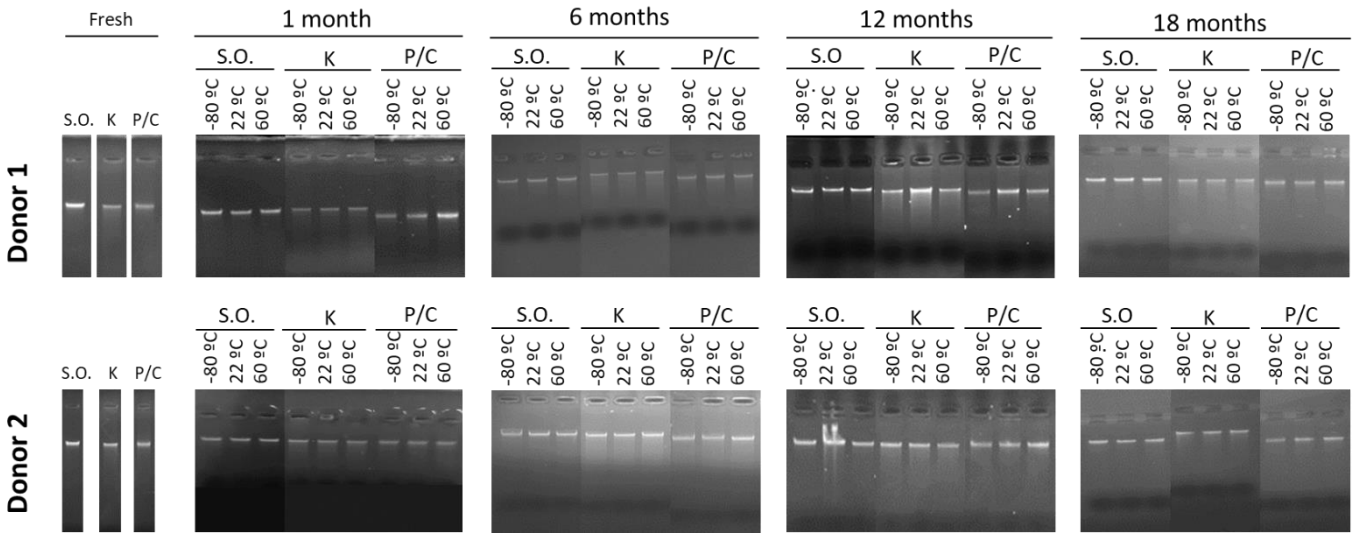

Figure 3. DNA integrity assessed by agarose gel electrophoresis. DNA of the 6 samples (2 donors and 3 extraction methods) was loaded in a 0,8% agarose gel electrophoresis to test their integrity during the storage time (1, 6, 12 and 18 months) under different conditions (-80 ºC, 22 ºC and 60 ºC). The results of the fresh DNA are also shown. None of the samples show significant signs of degradation. S.O.: Salting out; K: QIAamp DNA blood maxi kit; P/C: Phenol/Chloroform. For the simulated RT conditions (60 ºC) storage for 1, 6, 12 and 18 months corresponds to approximately 1, 6, 13 and 20 years at RT.

starting with a median DIN value ± SD of 8.37 ± 0.63 in fresh and a median DIN value ± SD of 8.40 ± 0.20 after 18 months at 60 ºC (Figure 2C and 2D). After optimum DIN values were obtained, we performed a multiplex long PCR to assess DNA functionality. As shown in Figure 4, agarose gel score using the PCR products from DNA purified with the QIAamp DNA blood maxi kit reported a 17.5 kb band which was distinguishable even in DNA stored for 18 months at 60 ºC, as well as in samples stored at -80 ºC and 22 ºC for the same period of time, giving a value of functionality and integrity of 9.5. In the case of DNA stored at 60 ºC, although a decrease in intensity of the 17.5 kb band is observed, and it may indicate a slight loss of integrity, it must be considered that the samples are subjected to extreme conditions to assess long-term results.

WES. The Quality summary of WES samples had a median read length of 100 bp and an average coverage of 100X. The accuracy of germline variant calling was evaluated in samples after different timepoints of real and simulated RT storage conditions. All benchmarked conditions (and their combinations) showed analogous Precision, Recall and F1-score values when comparing each timepoint with -80 ºC "gold standard" storage (Table 1). Even after 12 months at 60 ºC (which corresponds to approximately 13 years at RT), samples

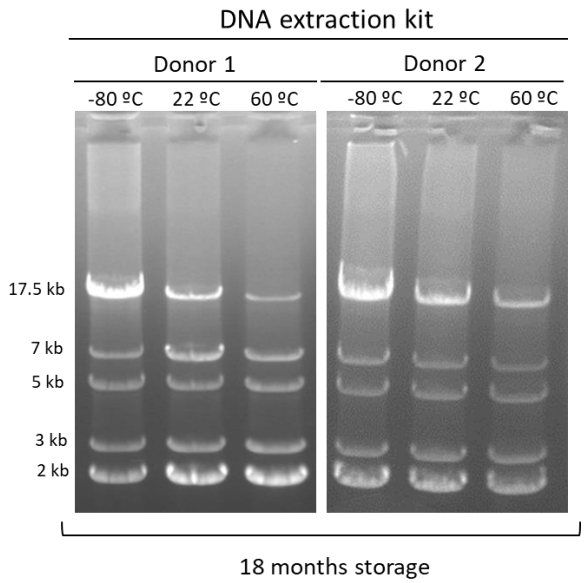

Figure 4. DNA integrity and functionality assessed by multiplex long PCR. DNA extracted using the Qiagen QIAamp DNA blood maxi kit from both donors and stored for 18 months at -80 ºC, 22 ºC and 60 ºC (equivalent to approximately 20 years at RT) was subjected to a multiplex long PCR. The products were then analyzed by 0,8% agarose gel electrophoresis and in all storage conditions the 17.5 kb band was distinguishable.

Table 1. WES variants detected in dried samples of 2 donors stored at RT (22 ºC) and 60 ºC (equivalent to 13 years at RT) compared to a pool of different aliquots of the same samples frozen and stored at -80 ºC ("gold standard") for different times. All DNA samples used for this study were extracted by Salting out. True positive (TP) and false negative (FN) position and genotype are the ones that match the reference variants on the reference sample ("gold standard"), or are not shared with the stored sample, respectively. Different time points of dried storage were compared with -80 ºC storage for Precision, Recall, and F1 scores.

Donors	*Time points*	*Variants detected*	*TP genotype*	*FN genotype*	*Precision*	*Recall*	*F1 score*
Donor 1	1 Day RT (22 ºC)	118482	109492	1252	0,92	0,99	0,96
110744 variants detected	1 Month RT (22 ºC)	120135	110001	743	0,92	0,99	0,95
at -80 ºC or "gold	1 Year RT (22 ºC)	118505	108809	1935	0,92	0,98	0,95
standard"	1 Year 60 ºC (13 years at RT)	120331	109668	1076	0,91	0,99	0,95
Donor 2	1 Day RT (22 ºC)	118382	107895	1238	0,91	0,99	0,95
109133 variants detected	1 Month RT (22 ºC)	119754	108326	807	0,90	0,99	0,95
at -80 ºC or "gold	1 Year RT (22 ºC)	119764	108059	1074	0,90	0,99	0,94
standard"	1 Year 60 ºC (13 years at RT)	120176	108200	933	0,90	0,99	0,94

showed very close values in the same metrics that at day 1 (Table 1), almost without variation in the F1-score, Recall and Precision values[7]. All the measurements of the real and simulated RT storage samples showed a recall of 99% in all time points analyzed, when compared with the -80 ºC "gold standard".

SNP arrays. Genome-wide analysis using high-density SNP arrays provided characterization of the donor samples through the identification of copy number (CN) changes and loss of heterozygosity (LOH) for millions of SNPs. For donor 1, CytoScan 750K array showed identical profiles between fresh DNA (Figure 5A) and DNA stored 1 year at -80 ºC (Figure 5B), 1 year at 22 ºC (Figure 5C) or 1 year at 60 ºC (Figure 5D). In addition, the CN profile revealed a small gain of 522 kb in the short arm of Chromosome 11 (11p15.4), which was observed in all the samples (Figure 5E) and corresponds to a codifying zone that contains CNGA4, CCKBR and CAVIN3 (OMIM genes). For donor 2, no alterations were detected on codifying DNA, but some regions showed alterations on non-codifying DNA, and equal CN profiles were found for all storage samples (data not shown).

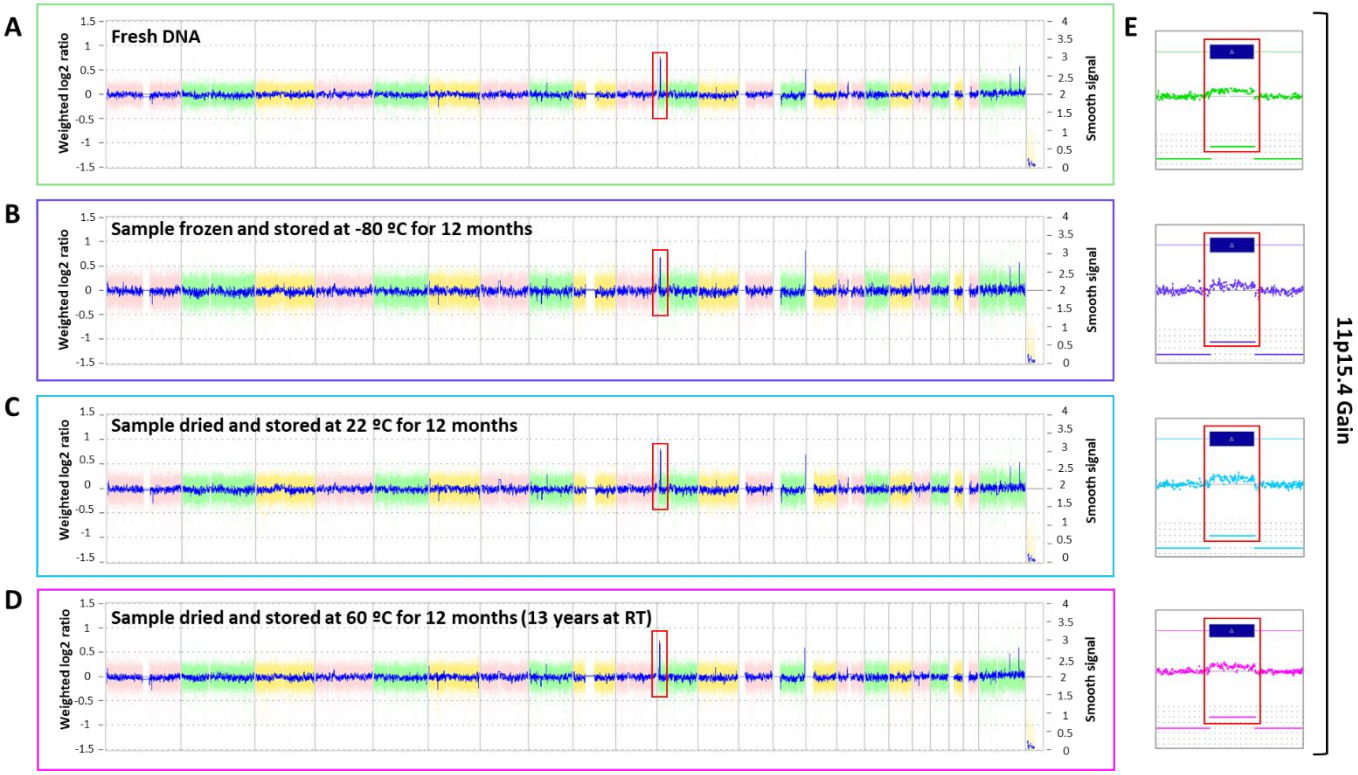

Figure 5. Cytogenetic analysis using SNP arrays from DNA of donor 1. The Array CGH CytoScan® 750K (Affymetrix, ThermoFisher Scientific, Waltham, MA, US) analysis was performed with DNA purified by salting out in fresh and stored aliquots for 1 year in different conditions. Genome-wide analysis showed similar copy number (CN) profiles for the fresh aliquot (A) and the aliquots stored at -80 ºC (B), 22 ºC (C) and 60 ºC, which is equivalent to approximately 13 years of RT storage (D). Highlighted with red rectangles, a small gain in the short arm of chromosome 11 (11p15.4) was observed in all the aliquots analyzed (A-E).

Discussion

Different attempts of dry preservation of biological materials have been made in the last decades with different degrees of success[8,9,10]. Although for some of these types of samples it has been demonstrated that it is possible the preservation at RT for at least 30 years with currently available methods[11], the main problem identified is the lack of global standards in the pre-analytical treatment of sample preservation, drying conditions and an efficient workflow that allows researchers to obtain and maintain high quality samples throughout an unlimited period of time where the scientist may recall a sample for further research[12-16].

300K Solutions has developed a methodology that aims to offer a standardized stabilization procedure for biological materials that allows their storage at RT perfectly integrable in a laboratory or biobank workflow. This work aims to provide a proof of concept of such technology applied to the storage of DNA samples and establish a starting point for its expansion to the storage of other biological materials at RT.

At present, many different -omics applications are used for precision medicine with stored biospecimens[17]. Specifically, for Molecular Biology applications, it is crucial that DNA shows an optimal integrity. This is more evident in samples which, in some cases, have been stored for years and, therefore, the integrity may be compromised.

Based on the global results obtained with the different techniques we tested in dried and stored DNA under real and simulated RT conditions, compared with the standard -80 ºC storage, it is clear that the technology here proposed could preserve the integrity of these samples for an unlimited time if stored under appropriate conditions. This may enormously contribute to Biobank management, changing the paradigm of the ULT storage, which currently has huge costs from an economic, energetic and space point of view with risky conditions for valuable collections.

Overall, the optimal performance of this technology in these preliminary tests, brings a promising solution for RT storage. Standardization in biobanks for QC criteria, preservation and storage processes are needed, and this new technology may contribute to this achievement.

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