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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 DNA is ultra-low temperature (ULT) freezing at -80 °C. However, maintaining those protective conditions implies high maintenance costs, large spaces, constant energy supply and safety measures to minimize the risk of missing collections.

Here, we propose an alternative approach for room temperature (RT) preservation based on DNA drying and stabilization that has the potential to be a real alternative to the current ULT DNA storage.

Objetive

Evaluate the quality, integrity, functionality, and applicability in genomic studies of DNA stabilized, dried and stored for different periods of time, under real (22 °C) and simulated (60 °C to recreate accelerating aging conditions based on the Arrhenius reaction rate function) RT conditions, and comparing them with those of DNA stored under "gold standard" conditions (frozen at -80 °C).

Materials and Methods

Peripheral blood of 2 healthy donors were obtained from Biobanco del Centro de Hemoterapia y Hemodonación de Castilla y León (CHHCyL). DNA was obtained from these samples using three methods: Salting out, Phenol/Chloroform and QIAamp DNA Blood Maxi Kit. 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 °C (control) and dried at RT (22 °C) or 60 °C (accelerating aging conditions).

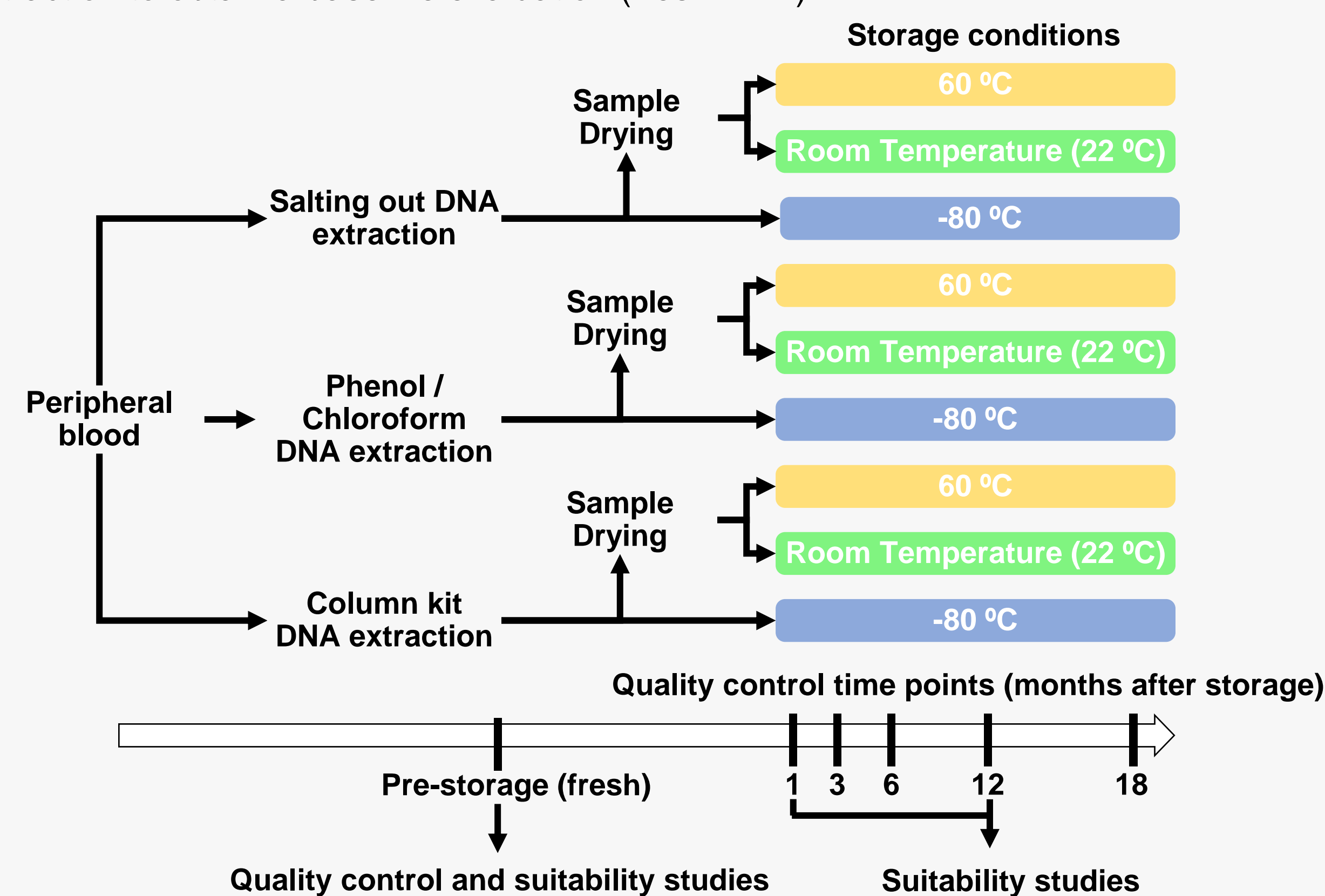
Then, after 1, 6, 12 and 18 months (equivalent to 1, 6, 13 and 20 years at RT for the aliquots stored at 60 °C) one aliquot of each condition was recovered by either thawing (samples stored at -80 °C) or rehydrating with Milli-Q H₂O (samples stored at 22 °C and 60 °C). Each aliquot material was then subjected to a basic quality control (QC) procedure according to the standards of Banco Nacional de ADN (BNADN) :

- DNA quantity and purity by spectrophotometry.
- Determination of DNA integrity by agarose gel electrophoresis.
- DNA integrity analysis by Agilent 2200 TapeStation System
- DNA functionality and integrity by multiplex long PCR.

Finally, 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 NIM Genetics, 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).

Importantly, each sample was submitted to a QC and suitability assessment just after extraction to obtain a baseline evaluation (fresh DNA).



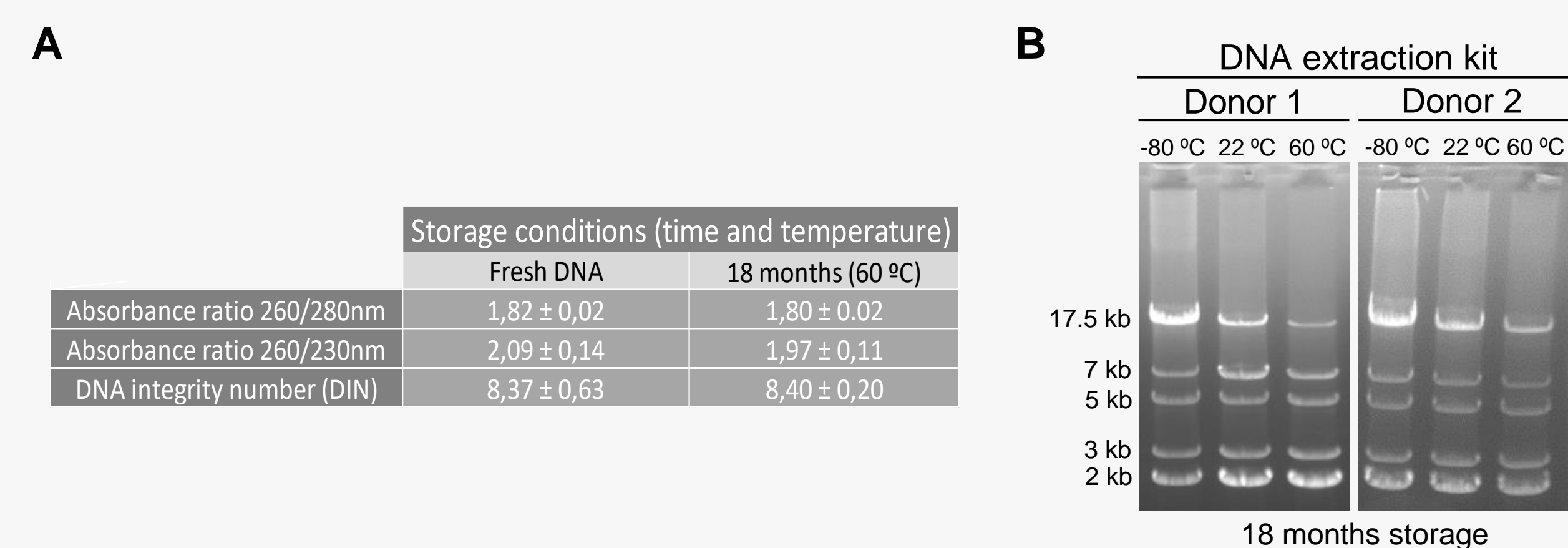
Conclusions

- The technology here proposed could preserve the integrity of DNA samples for an unlimited time if stored under appropriate conditions.
- 300K Solutions has developed a methodology that aims to offer a standardized stabilization procedure for biological materials that allows their storage at RT.

Results

First, to assess DNA purity and integrity we compared absorbance ratios 260/280 and 260/230 and the DNA integrity number (DIN), respectively, in all samples stored under different storage conditions at different time points and observed that samples stored 18 months at 60 °C (equivalent to 20 years at RT) showed the same quality that fresh DNA (A).

After optimum DIN values were obtained, we performed a multiplex long PCR to assess DNA functionality and agarose gel score using the PCR products from DNA purified with the QIAamp DNA blood maxi kit reported a 17.5 kb band in all samples, giving a value of functionality and integrity of 9.5 out of 10 (B).



Finally, we evaluated the adequacy of DNA stored at RT for its use in genomic studies using to different approaches. First, we compared WES variants detected in dried samples of 2 donors stored at RT (22 °C) and 60 °C (equivalent to 13 years at RT) to a pool of different aliquots of the same samples frozen and stored at -80 °C ("gold standard") for different times and observed that 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 °C "gold standard" (C).

Second, we performed Cytogenetic analysis using SNP arrays that showed similar copy number (CN) profiles for the fresh aliquot and the aliquots stored at -80 °C, 22 °C and 60 °C for 1 year in both donors. Furthermore, in the case of donor 1, the CN profile revealed a small gain of 522 kb in the short arm of Chromosome 11 (11p15.4) in all samples (D; only data from donor 1 is shown).

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

