Use of Freeze-Dried Cell Lines as Controls for Next-Generation Sequencing Studies

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Introduction

The rise of Next-Generation Sequencing (NGS) technologies has had a profound impact in the study of hematological neoplasias, leading to a better understanding of these malignancies. However, there is a lack of standardization in terms of quality control to ensure the use of these techniques in the clinical field. In this context, it is extremely important the availability of samples with known biomarkers and alterations to be used as controls in these genomic studies. Moreover, these samples should have long-term stability and its analysis must be replicable, both through time and in different laboratories.

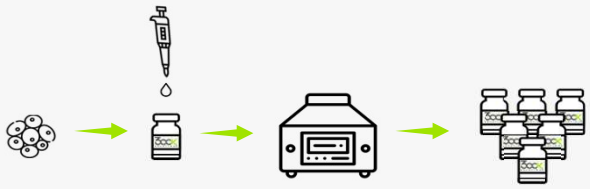
To face this challenge, 300K Solutions is developing a disruptive and innovative technology to allow room temperature (RT) storage of cell lines currently used as controls in NGS studies. Thus, this type of storage could potentially guarantee the standardization needed for the use of these cutting-edge techniques in clinical diagnostics.

Objetive

- To evaluate the purity, integrity and functionality of the genetic material extracted from freeze-dried cell lines.
- To assess the applicability of freeze-dried cell lines as controls for NGS studies.

Materials and Methods

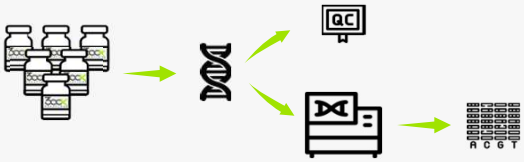
Four different lymphoproliferative disorders (LPDs) cell lines (H929, CA46, RS4;11 and REH) were cultured and aliquots of 1.000.000 or 500.000 cells were obtained and freeze-dried.



Using the phenol/chloroform extraction method, DNA from freeze-dried aliquots of each cell line was obtained and subjected to a basic quality control (QC) assessment following the Proficiency Standards stablished by the International Society for Biological and Environmental Repositories (ISBER):

- DNA purity by spectrophotometry.
- Double-strand DNA quantity by fluorimetry.
- Determination of DNA integrity by agarose gel electrophoresis.
- Functionality and DNA integrity by multiplex long PCR.

Finally, to assess the suitability of the dried samples as controls for NGS studies we performed the EuroClonality-NGS DNA capture (EuroClonality-NDC) assay (Univ8 Genomics, Belfast, Northern Ireland).



Conclusions

- The technology here proposed offers protection during the freeze-drying process of cell lines resulting in long-term stability at RT.
- DNA extracted from freeze-dried cell lines has an optimal quality that allows its use for genomic studies.
- 300K Solutions has developed a methodology that aims to offer the use of freeze-dried samples as controls for NGS studies, potentially providing the material required for the standardization of these techniques to ensure its clinical application.

Results

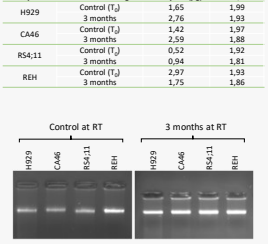
To evaluate if our stabilization technology allowed long-term stability during RT storage of freeze-dried cell lines (aliquots of 500.000 cells), we compared DNA quality right after the freeze-drying process and after 3 months of RT storage. This QC assessment is focussed on 4 different parameters: purity, DNA yield, integrity and functionality.

First, we assessed DNA purity by spectrophotometry and observed that all four cell lines showed A260/280 ratio values above 1.6 even after 3 months of RT storage, which indicates absence of protein contamination. Moreover, double-strand DNA quantification by fluorimetry confirmed that we were able to extract enough DNA for further experiments (at least 0,5µg) (A).

Second, using agarose gel electrophoresis we confirmed DNA integrity in all samples (B), which we further assessed together with DNA functionality, by multiplex long PCR. All samples displayed a 17.5 kb band (C), confirming that our stabilization technology assures DNA functionality after long-term RT storage.

A

Sample ID	Time of RT storage	DNA yield (µg)	A260/280
H929	Control (T_0)	1,65	1,99
	3 months	2,76	1,93
CA46	Control (T_0)	1,42	1,97
	3 months	2,59	1,88
RS4;11	Control (T_0)	0,52	1,92
	3 months	0,94	1,81
REH	Control (T_0)	2,97	1,93
	3 months	1,75	1,86



Once we confirmed that our stabilization solution allows optimal quality DNA extraction, we decided to assess its suitability for NGS studies. Specifically, we performed the EuroClonality-NDC assay (Univ8 Genomics, Belfast, North Ireland) which is used for the study of LPDs. The reason why we choose this genomic assay is because it uses as validation samples, among others, the four cell lines we have tested.
The metrics obtained in this NGS analysis using DNA from the H929 cell line were within the stablished acceptance criteria. Moreover, the results (shown in the table below) were the expected for this specific cell line.

Cell line	Locus	Rearregement	Structural variations (SV)	Copy number alterations (CNA)
H929	TRB	TRBD1>TRBJ2-2	t(4;14) NSD2(MMSET)/IGHswc t(8;20) MYC/FAM242A	Amp/Gain: CKS1B, 11q, BCL2, TCF3, 20q Del/Loss: TENT5C, NOTCH2, BCL6*, CARD11, chr9, ERG2, WT1, KRAS, 13q, PCLG2*
	IGH	IGHD1-1>IGHJ3		
	IGH	IGHV3-9>IGHJ5*		
	IGK	IGKV3-15>IGKJ1		

* Alterations not detected in this analysis.

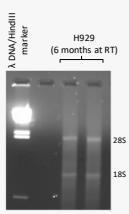
Finally, we wanted to assess if our stabilization solution could allow the extraction of high-quality RNA after RT storage.

In this sense, preliminary results using freeze-dried aliquots of the cell line H929 (1.000.000 cells each) stored at RT for 6 months showed good-quality RNA extraction in terms of purity (D) and integrity (E). Nevertheless, further experiments are needed to evaluate a potential used of this genetic material in more complex genomic studies.

D

Sample ID	Time of RT storage	RNA yield (µg)	A260/280
H929	6 months	2,04	1,91

E



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