APPLICATION NOTE

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

Using 300K Solutions SL Cell Line Stabilization Solution

► Introduction

The rise of Next-Generation Sequencing (NGS) technologies has had a profound impact in the study of hematological malignancies, 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.

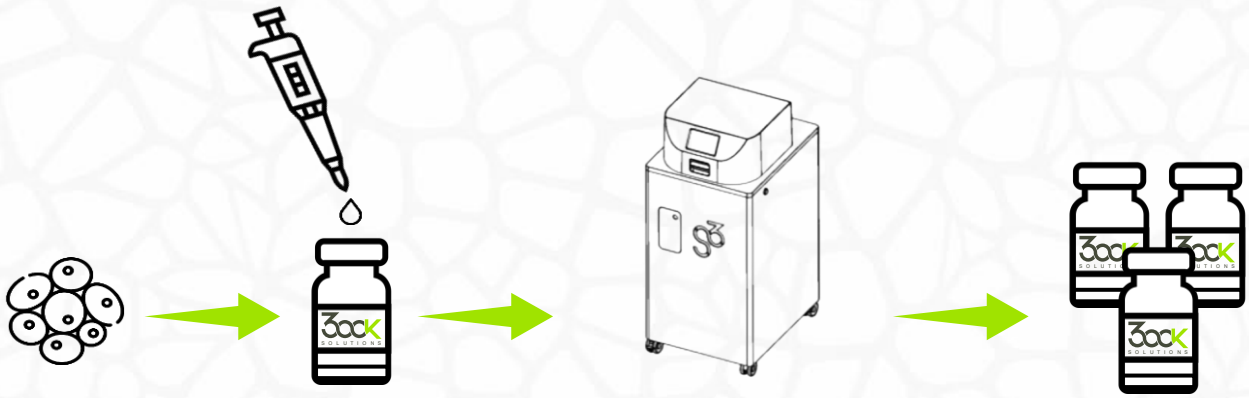
In this context, it is extremely important the availability of samples with known biomarkers and alterations to be used as controls in these genomic studies. Moreover, these samples should have long-term stability and its analysis must be replicable, both through time and in different laboratories.

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.

► Materials and methods

Four different lymphoproliferative disorders (LPDs) cell lines (H929, CA46, RS4;11 and REH) were cultured and aliquots of $5x10^5$ cells were obtained and freeze-dried (also called lyophilized) using the stabilization solution developed by *300K Solutions SL.*

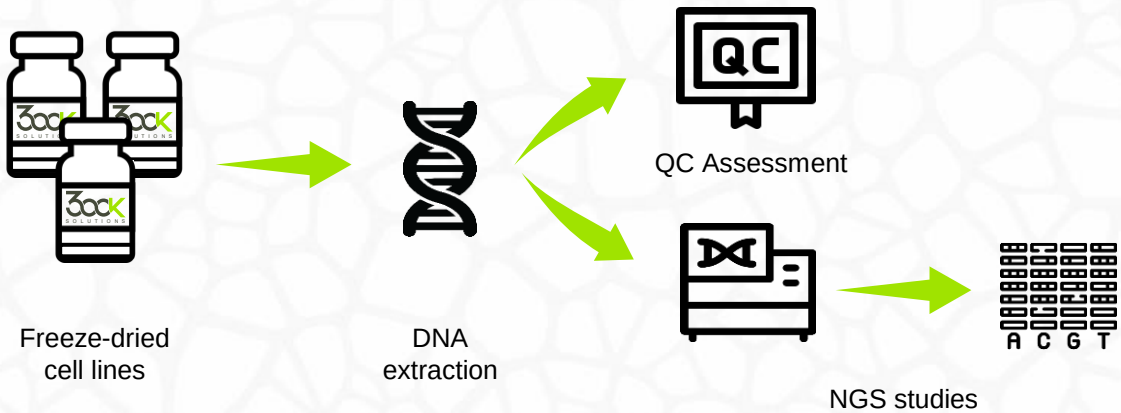
Workflow



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).



► Results

QC assessment was performed in cell line samples stored at room temperature (RT) for up to 3 months. This quality control focused on double strand DNA quantification and purity (Table 1), as well as on DNA integrity and functionality by agarose gel electrophoresis (A) and multiplex long-PCR (B), respectively.

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

Table 1. DNA purity and dsDNA quantification in freeze-dried cell lines right after the lyophilization process and after 3 months of RT storage.

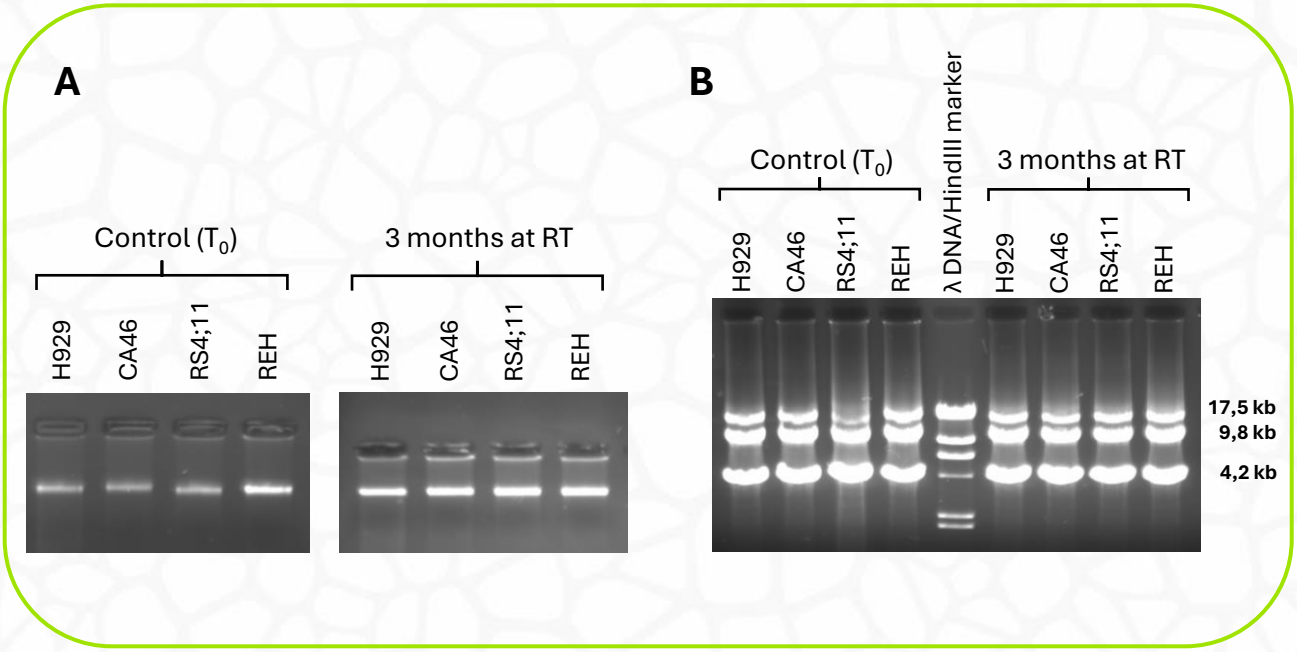


Figure 1. DNA integrity (A) and functionality (B) assessment in freeze-dried cell lines right after the lyophilization process and after 3 months of RT storage.

► Results

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 all four cell lines (stored 1 year at RT) were within the stablished acceptance criteria. Moreover, the results were the expected for these specific cell lines (Table 2; results regarding SNVs, even though not shown, were also similar in all four cell lines).

Sample ID	Locus	Rearregement	Stuctural variations (SV)
H929	TRB	TRBD1>TRBJ2-2	t(4;14) NSD2(MMSET)/IGHswc t(8;20) MYC/FAM242A
	IGH	IGHD1-1>IGHJ3	
	IGH	IGHV3-9>IGHJ5*	
	IGK	IGKV3-15>IGKJ1	
CA46	IGH	IGHD3-9>IGHJ4	Tt(8;14) MYC/IGHswc
	IGH	IGHV5-51>IGHJ4	
	IGK	IGKV2-28>IGKJ2	
REH	TRB	TRBV27>TRBD2	t(1;2) HYDIN2/IGKV
	TRD	TRDV2>TRDD3	
	TRB	TRBV20-1>TRBJ2-7	
	TRA+D	TRDV2>TRAJ29	
	TRA+D	TRDV2>TRAJ61	
	TRG	TRGV4>TRGJ2	
	TRG	TRGV9>TRGJ2	
	IGH	IGHV3-15>IGHJ6	
	IGK	IGKV2-29>IGKJ4	
	IGL	IGLV2-8>IGLJ2=IGLJ3	
	IGL	IGLV3-21>IGLJ2=IGLJ3	
	IGK	IGKV3-20>Kde	
	IGK	intron>Kde	
RS4;11	TRA+D	TRDV2>TRAJ53	t(4,11) AFF1/KMT2A
	TRA+D	TRDV2>TRAJ29	
	IGH	IGHV3-9>IGHJ5	
	IGH	IGHV3-20>IGHJ5	
	IGK	IGKV4-1>IGKJ1	
	IGL	IGLV4-3>IGLJ2=IGLJ3	
	IGK	IGKV7-3>Kde	

* Alterations not detected in this analysis.

Table 2. Expected results using EuroClonality-NDC in four different LPD cell lines used as proficiency samples for this assay.

► Conclusions

1. The technology here proposed offers protection during the freeze-drying process of cell lines resulting in long-term stability at RT.

2. DNA extracted from freeze-dried cell lines has an optimal quality that allows its use for genomic studies.

3. 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.

►References

Validation of the EuroClonality-NGS DNA capture panel as an integrated genomic tool for lymphoproliferative disorders (doi: 10.1182/bloodadvances.2020004056).

EuroClonality-NDC Assay: Quick Reference Guide (released September 2021)

Images: *Flaticon*