Development and Testing of Novel Technology for Generation of Lyophilised Cell Lines for Use as Controls for Next-Generation Sequencing Assays

J.P. Stewart[1], M.G. Álvarez[2], A. Hernández[2], M.E. Sarasquete[3], D. Gonzalez[1], M. Martín-Ayuso[2]

[1]*Patrick G Johnston Centre for Cancer Research, Queens University Belfast, Belfast, United Kingdom.*
[2]*300K Solutions, Salamanca, Spain.*
[3]*Department of Hematology, University Hospital of Salamanca (HUSA/IBSAL), CIBERONC, CIC-IBMCC (USAL-CSIC), Salamanca, Spain.*

Introduction

- International Consensus Classification (ICC) and World Health Organization (WHO) classification of lymphoid neoplasms highlights the increasing role of genomics in accurate classification and prognosis of lymphoproliferative disorders (LPDs).
- WHO and ICC classifications require laboratories to adopt multi-gene testing, such as Next Generation Sequencing (NGS), to simultaneously examine clonal IG/TCR rearrangements, structural variants, mutations and copy number alterations.
- However, there is a lack of reference materials (RM) with known specific genomic alterations relevant to LPD to monitor performance of molecular testing techniques. Moreover, RM should exhibit long-term stability and reproducibility, be readily available and compatible with laboratory workflows.
- To address this challenge, 300K Solutions (Salamanca, Spain) have developed an innovative technology enabling room temperature (RT) storage of extensively characterized lyophilized LPD cell lines for use as RM in NGS studies.

Objectives

1. To evaluate the yield and integrity of genomic DNA (gDNA) extracted from lyophilized LPD cell lines for use as RM for NGS studies.
2. To determine use of extracted gDNA as reference material for the EuroClonality-NDC assay

Materials and Methods

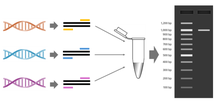
1 Cell culture of four LPD cell lines (H929, CA46, RS4;11 & REH)

2 Aliquot preparation (5 x10^5 cells resuspended in stabilization buffer)

3 Freeze-dry and store for 1 year at room temperature

4 Extraction of genomic DNA using Promega Maxwell 16 LEV Blood DNA kit on the Promega Maxwell 16

5 QC of gDNA using Qubit and Agilent gDNA ScreenTape Assay on the TapeStation 4200



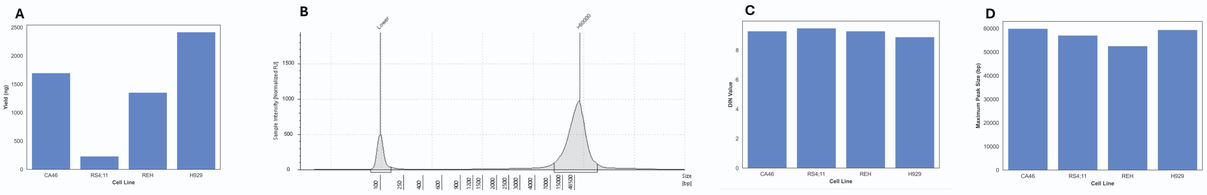
6 gDNA integrity assessment using multiplex long PCR

7 Preparation of NGS libraries using AVENIO Edge and manual target enrichment

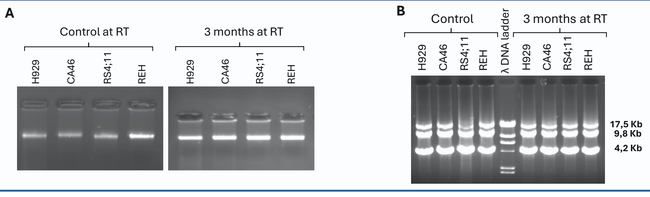
8 Sequencing on NextSeq 500 Mid Output Kit v2.5 (150 Cycles) followed by processing of FASTQ files on EuroClonality-NDC app

Results

To evaluate if the stabilization technology allowed long-term stability during RT storage of freeze-dried cell lines (aliquots of 5 x 10^5 cells), we compared DNA quality right after the freeze-drying process and after 12 months of RT storage. This QC assessment is focussed on 4 different parameters: DNA yield (A) and DNA integrity (B, C &D). Results indicate sufficient DNA yield is extracted for multiple assays with excellent DNA integrity representative of high molecular weight DNA.



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

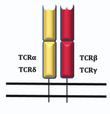


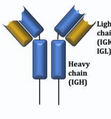
Samples underwent library preparation, hybridisation and sequencing using the EuroClonality-NDC assay. Initial sequencing metrics for all samples passed acceptance criteria with low PCR duplicates percentages (20-22%), unique mean target coverage depth (1386x-1620x) and on-target read percentages ranging from 78-80%).

Cell Line	Total Paired End Reads	Duplicated Reads (%)	Unique mean target coverage	Bases on or near target (%)
CA46	9,277,406	19.8	1389	78.2
H929	9,296,709	20.9	1407	80.1
REH	9,297,214	20.7	1386	78.1
RS4;11	10,868,398	21.8	1620	78.9

Finally we examined output files from the EuroClonality-NDC bioinformatics pipeline. IG/TCR rearrangement profile (A), structural variant (B) and mutation profiles (C) showed excellent concordance with benchmark data.

A





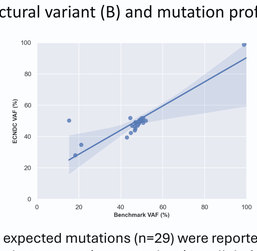
- 17/17 (100%) expected IG rearrangements were detected across the 4 cell lines.
- 10/10 (100%) expected TCR rearrangements were detected across the 4 cell lines.

B

Cell Line	Expected Translocation	Detected
CA46	t(8;14) MYC/IGHswc	✓
H929	t(4;14) MMSET/IGHswc	✓
H929	t(8;20) MYC/FAM242A	✓
REH	t(1;2) HYDIN2/IGKV	✓
RS4;11	t(4;11) AFF1/KMT2A	✓

- The correct structural variant was detected in each cell line and confirmed using IGV analysis of the BAM file.

C



- All expected mutations (n=29) were reported with good agreement in reported variant allele frequency

Conclusions

- The novel technology used to generate lyophilised cell lines leads to a product with demonstrable stability over 12 months.
- gDNA extracted from the lyophilised cell lines had sufficient yield for multiple assays with excellent gDNA integrity making it suitable for NGS-based assays.
- Well characterised lyophilised LPD cell lines provide a straightforward solution for adoption as RM to encompass the entire laboratory workflow from extraction through to sequencing in laboratories utilising the EuroClonality-NDC assay.

EuroClonality