Development of a fully automated workflow for integrated genomic profiling of haematological malignancies

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Introduction

Recent recommendations highlight the increasing role of genomics in accurate classification, prognosis and risk-stratification of haematological malignancies.

Such holistic classifications require laboratories to adopt comprehensive genomic profiling protocols using Next Generation Sequencing (NGS). NGS workflows are laborious and time-consuming, requiring significant staff resources (9-10 hours within a 2-day workflow) while also requiring significant technical expertise to ensure reproducible intra-assay and inter-assay performance.

The AVENIO Edge System (Roche Sequencing Solutions, CA, US) is a unique automated instrument with the capability of performing library preparation (LP), quantification, pooling and target enrichment (TE) in less than 24h with no manual input.

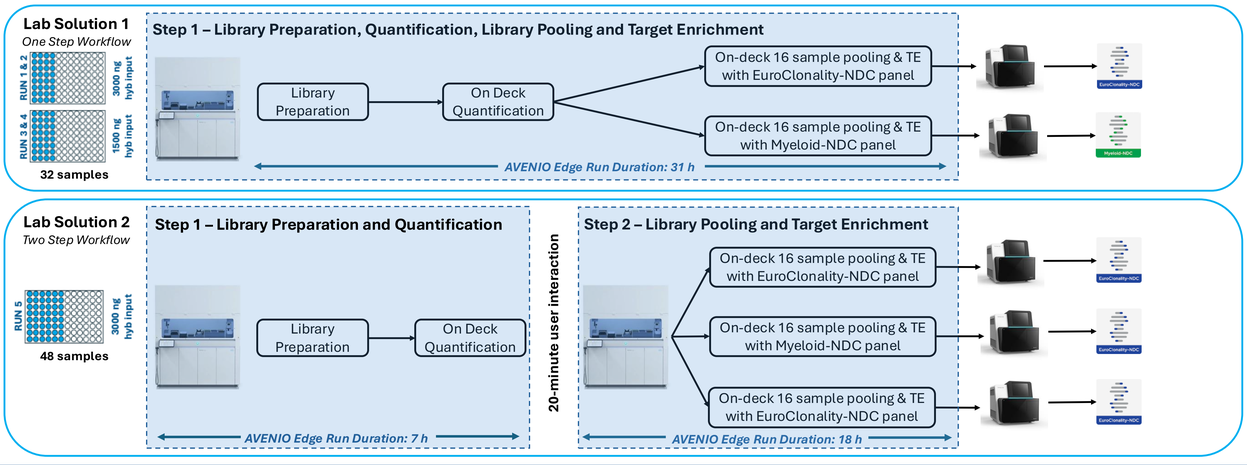
The EuroClonality-NDC assay (Univ8 Genomics, Belfast, UK) has been validated to simultaneously detect IG/TCR rearrangements, structural variants, mutations and copy number alterations in a large series of lymphoid malignancies.

The Myeloid-NDC assay (Univ8 Genomics, Belfast, UK) can detect fusion genes, mutations and copy number alteration in all myeloid disorders. We propose an integrated solution for genomic profiling of all hematological malignancies using a “single click” walk-away protocol.

Objectives

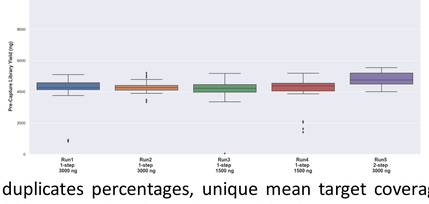
1. To develop a fully automated solution on the AVENIO Edge for the integrated genomic characterisation of heamatological malignancies.
2. To optimise the hybridisation step to ensure optimal target enrichment
3. To validate the method using a panel of haematological cell lines

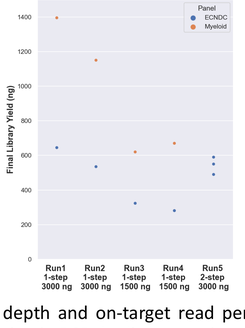
Materials and Methods

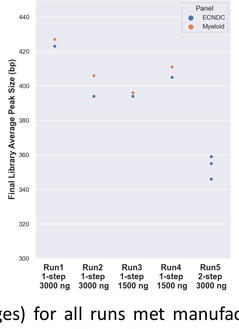


Results

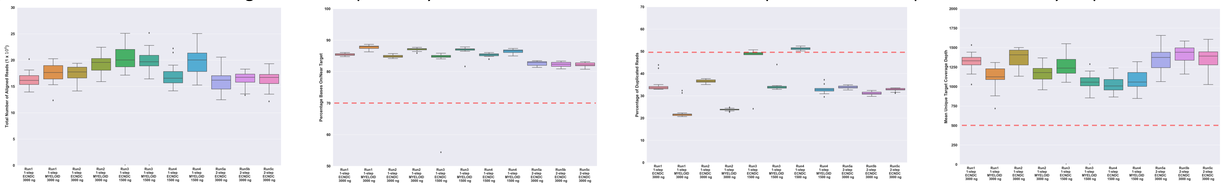
On deck DNA quantification demonstrated a reproducible yield of pre-capture libraries with just 100 ng DNA input. Following TE with the EuroClonality-NDC/Myeloid-NDC panel, final library yields were comparable to expected yields from manual library preparation. Fragmentation profile analysis of the final libraries also showed average fragment sizes within manufacturer guidelines.

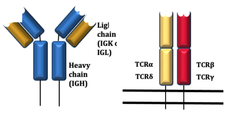






Sequencing metrics (PCR duplicates percentages, unique mean target coverage depth and on-target read percentages) for all runs met manufacturer’s specifications. Runs with 3000 ng total DNA input for hybridization showed a reduction in PCR duplicate rate in comparison to 1500 hyb input.



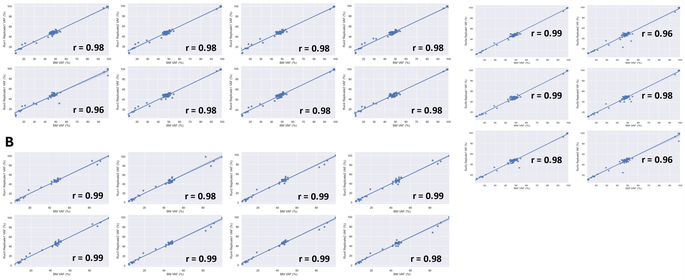


- 291/294 (98.8%) IG rearrangements were detected across all runs
- 364/364 (100%) TCR rearrangements were detected across all runs

149/150 (99.3%) structural variants (SV) detected across all runs

Run	Workflow	Panel	Hyb Input	Replicate	Expected SV	Observed SV	(%)
Run 1	1-step	ECNDC	3000	Replicate 1	9	9	100.0
				Replicate 2	9	9	100.0
Run 1	1-step	Myeloid	3000	Replicate 1	3	3	100.0
				Replicate 2	3	3	100.0
Run 2	1-step	ECNDC	3000	Replicate 1	9	9	100.0
				Replicate 2	9	9	100.0
Run 2	1-step	Myeloid	3000	Replicate 1	3	3	100.0
				Replicate 2	3	3	100.0
Run 3	1-step	ECNDC	1500	Replicate 1	9	8	88.9
				Replicate 2	9	9	100.0
Run 3	1-step	Myeloid	1500	Replicate 1	3	3	100.0
				Replicate 2	3	3	100.0
Run 4	1-step	ECNDC	1500	Replicate 1	9	9	100.0
				Replicate 2	9	9	100.0
Run 4	1-step	Myeloid	1500	Replicate 1	3	3	100.0
				Replicate 2	3	3	100.0
Run 5a	2-step	ECNDC	3000	Replicate 1	9	9	100.0
				Replicate 2	9	9	100.0
Run 5b	2-step	ECNDC	3000	Replicate 1	9	9	100.0
				Replicate 2	9	9	100.0
Run 5c	2-step	ECNDC	3000	Replicate 1	9	9	100.0
				Replicate 2	9	9	100.0

Mutation profiles showed excellent concordance between replicates and runs, as well as with existing data for ECNDC (A) and Myeloid (B) panels with 1-step approach and ECNDC panel with 2-step approach (C)



Conclusions

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The AVENIO Edge System combined with the EuroClonality-NDC and Myeloid-NDC assays provide a robust and fully automated solution for the integrated genomic characterization of all haematological malignancies that is compatible with current international recommendations.

This first-in-class solution provides a complete walk-away workflow operated with a single click, that can be implemented in any laboratory with no prior NGS experience required.