

Development of an automated genomic profiling assay for myeloid malignancies research

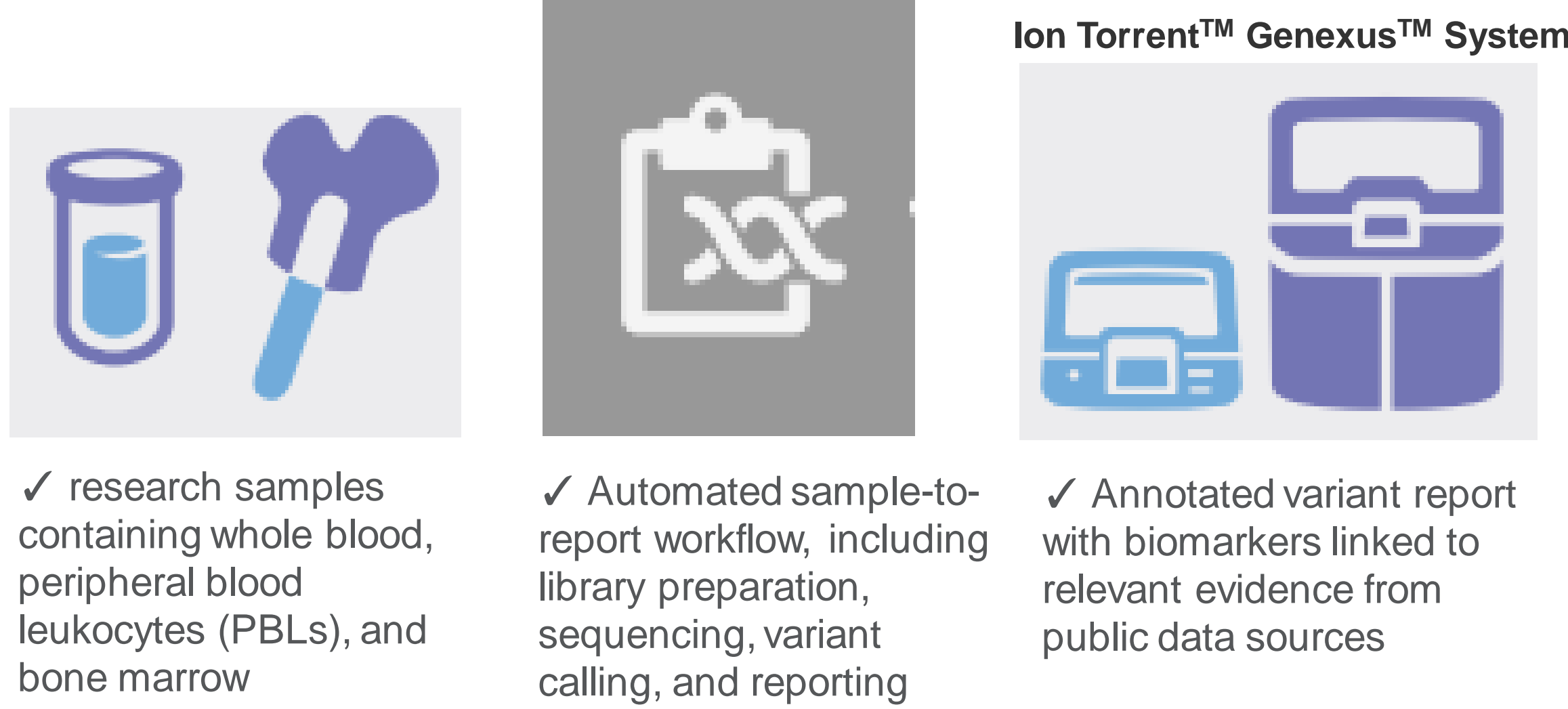
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INTRODUCTION

Myeloid malignancies are clonal disorders caused by genetic alterations in hematopoietic stem cells and myeloid progenitor cells. The major myeloid disorders include acute myeloid leukemia, myeloproliferative neoplasms, and myelodysplastic syndromes.

Previously, we developed the OncoPrint Myeloid Assay on the Ion GeneStudio™ S5 System to identify relevant alterations in myeloid malignancies. The assay covered 45 DNA genes, 38 fusion genes and 5 expression targets. We now describe the OncoPrint Myeloid Assay GX on the Genexus™ Integrated Sequencer with major performance improvements: an automated sample-to-report workflow with rapid turnaround time (TAT, < 24 hours) and minimal hands-on time (HoT).

Fig 1. Automated sample-to-report workflow in 1 day



MATERIALS AND METHODS

The new myeloid research assay was developed on the Genexus™ Integrated Sequencer that offers automated library prep, templating, sequencing, data analysis, and report generation. Libraries for DNA, RNA, and DNA-RNA were created and combined for templating on beads in an isothermal on chip amplification reaction. Library and templating conditions were optimized for improved performance in relevant targets and mutations, such as the CEBPA gene, which is GC-rich and difficult to amplify, and FLT3-ITDs, the length of which is a challenge to sequence alignment algorithms. The assay was tested using replicates of cell lines, commercial controls, and research samples from bone marrow aspirates and whole blood. For each sample, depending on the nucleic acid type, 20 ng of DNA and/or 10 ng of RNA was used as input.

RESULTS

The sequencing metrics of the assay demonstrated high (>98%) uniformity and consistent read depth (>2000x). Analytical sensitivity of > 97% and PPV of 100% was observed in research samples and control samples down to 2.5% VAF. The total nucleic acid-to-report TAT for 8 DNA and 8 RNA samples was 22.5 hours and the total HoT was 20 minutes.

Fig 2. Highlights of OncoPrint Myeloid Assay GX



OVERVIEW

Table 1. OncoPrint Myeloid Assay GX v2 gene targets

DNA				RNA												
Hotspot only (28)		Full CDS (17)		Fusion driver (30)		Gene expression (5)		Expression control (5)								
ABL1	SETBP1	ASXL1	ABL1	MET	BAALC	EIF2B1	ANKRD26	SF3B1	BCOR	ALK	MLL10	MECOM	FBXW2			
BRAF	SMC1A	CALR	BCL2	MYBL1	MYC	PSMB2	CBL	SMC3	CEBPA	BRAF	MYBL1	SMC1A	PUM1			
CSF3R	SRSF2	ETV6	CCND1	MYH11	WT1	TRIM27	DDX41	U2AF1	EZH2	CREBBP	NTRK3	DNMT3A	WT1	IKZF1	EGFR	NUP214
FLT3		NF1	ETV6	NUP98	GATA2	PHF6	FGFR1	PDGFRB	HRAS	PRPF8	FGFR2	PDGFRB	IDH1	RB1	FUS	RARA
IDH2		RUNX1	HMG2	RBM15	JAK2	SH2B3	JAK2	RUNX1	KIT	STAG2	KMT2A	TCF3	KRAS	TET2	(MLL-PTD)	TFE3
MPL		TP53	MECOM	MYD88	ZRSR2	NPM1	NRAS	PPM1D	PTPN11							

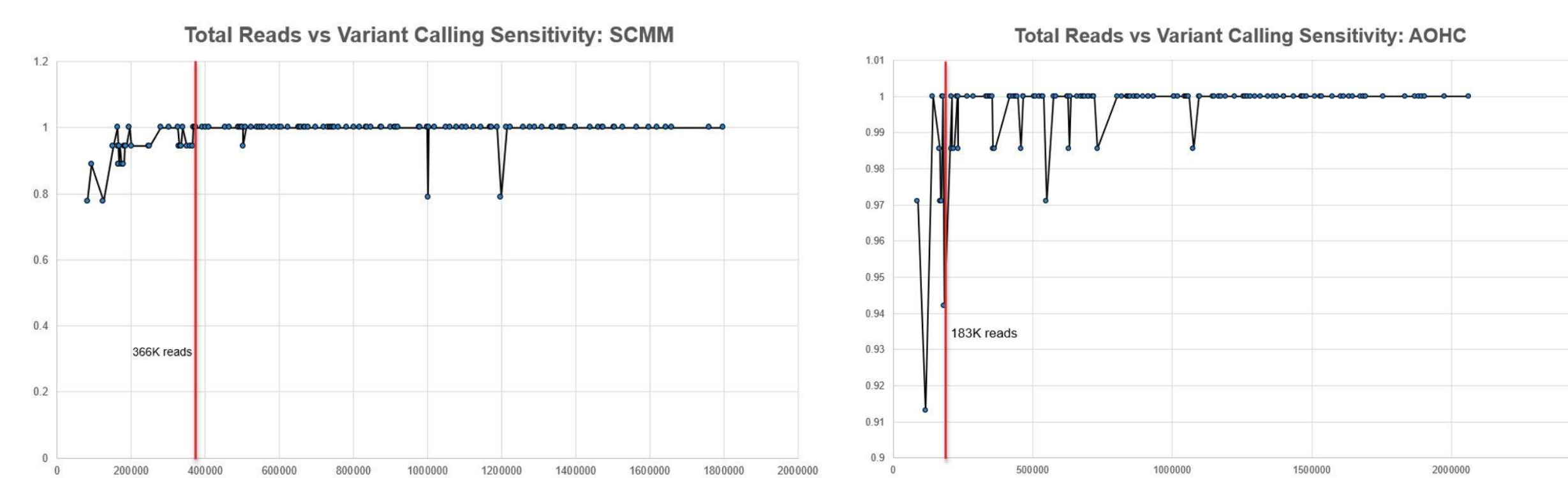
Table summarizes the target DNA and RNA gene content of the assay. With this research assay, you can profile key targets such as FLT3, TP53, NPM1, KIT, IDH1/2, JAK2, RUNX1, and PML-RARA, along with many other biomarkers that we've investigated using the latest research publications. In total, assay contains 45 DNA and 40 RNA genes.

Table 2. DNA variant calling performance with hotspot control, DNA reference standard, and Seraseq® Myeloid Mutation DNA Mix

Hotspot control (70 variants expected)			Seraseq® Myeloid Mutation DNA Mix (20 expected)			DNA reference standard (12 expected)		
Run #	TP detected	Sensitivity	Run #	TP detected	Sensitivity	Run #	TP detected	Sensitivity
Rep1	69	98.6%	Rep1	20	100%	Rep1	12	100%
Rep2	70	100%	Rep2	19	95.0%	Rep2	11	91.7%
Rep3	69	98.6%	Rep3	19	95.0%	Rep3	12	100%
Rep4	69	98.6%	Rep4	19	95.0%	Rep4	12	100%
Rep5	70	100%	Rep5	19	95.0%	Rep5	11	91.7%
Rep6	70	100%	Rep6	19	95.0%	Rep6	11	91.7%
Rep7	70	100%	Rep7	19	95.0%	Rep7	11	91.7%
Rep8	70	100%	Rep8	19	95.0%	Rep8	11	91.7%
Rep9	70	100%	Rep9	19	95.0%	Rep9	11	91.7%
Rep10	69	98.6%	Rep10	19	95.0%	Rep10	11	91.7%
Overall	696/700	99.4%	191/200	95.5%	113/120	94.2%		

The DNA variant calling performance was tested on 3 analytical controls, including AcroMatrix™ Oncology Hotspot Control (70 OncoPrint variants expected), Seraseq® Myeloid Mutation DNA Mix (20 OncoPrint variants expected), DNA reference standard (12 OncoPrint variants expected). OncoPrint Variants 5.16 filter was applied to filter for OncoPrint variants. 10 replicates of each sample were sequenced and variants including SNVs and InDels (including FLT3ITDs) were detected. The overall sensitivity was 98.0% among all the replicates of all the samples. Among all replicates of hotspot control 100% PPV was observed.

Fig 3. Sensitivity vs read depth in DNA variant calling



Sequencing results with control samples were downsampled to vary the number of mapped reads. With high uniformity of amplification 200K mapped reads in AOHC and 400K reads in Seraseq Myeloid Mutation Mix were required to support 95% Sensitivity.

Table 3. InDels detected in hotspot control, DNA reference standard, and Seraseq® Myeloid Mutation DNA Mix

(A) Hotspot control									
Genes	Expected VAF	AA Change	AOHC 100% sample input rep1	AOHC 100% sample input rep2	AOHC 100% sample input rep3	MegaMix AF ~5% rep1	MegaMix AF ~5% rep2	MegaMix AF ~2.5% rep1	MegaMix AF ~2.5% rep2
ABL1	15-35%	p.Ile293delinsMetPro	9.6%	10.11%	7.84%	2.53%	2.14%	-	-
FLT3	5-15%	p.Phe594_Asp600dup	4.39%	4.01%	4.93%	3.37%	4.47%	2.88%	2.02%
KIT	5-15%	p.Ala502_Tyr503dup	6.86%	5.36%	7.9%	4.22%	5.45%	2.48%	3.09%
RB1	5-15%	p.Leu676PheTsr16	7.08%	7.44%	6.71%	7.05%	6.92%	2.3%	3.13%

(A) Multiple replicates of hotspot control with different dilutions were tested, including 3 replicates of 100% sample input (i.e. no dilution), 2 replicates of MegaMix samples with targeted Allele Frequency (AF) at 5%, and 2 replicates of MegaMix samples with targeted AF at 2.5%. (MegaMix sample contains the same variants in hotspot control except that the expected AF for all of them are 50%. A 5% AF dilution is 10-fold dilution of the original sample, and a 2.5% AF dilution is 20-fold dilution of the original sample.) There are 4 InDels that were detected in AOHC as low as about 2% AF.

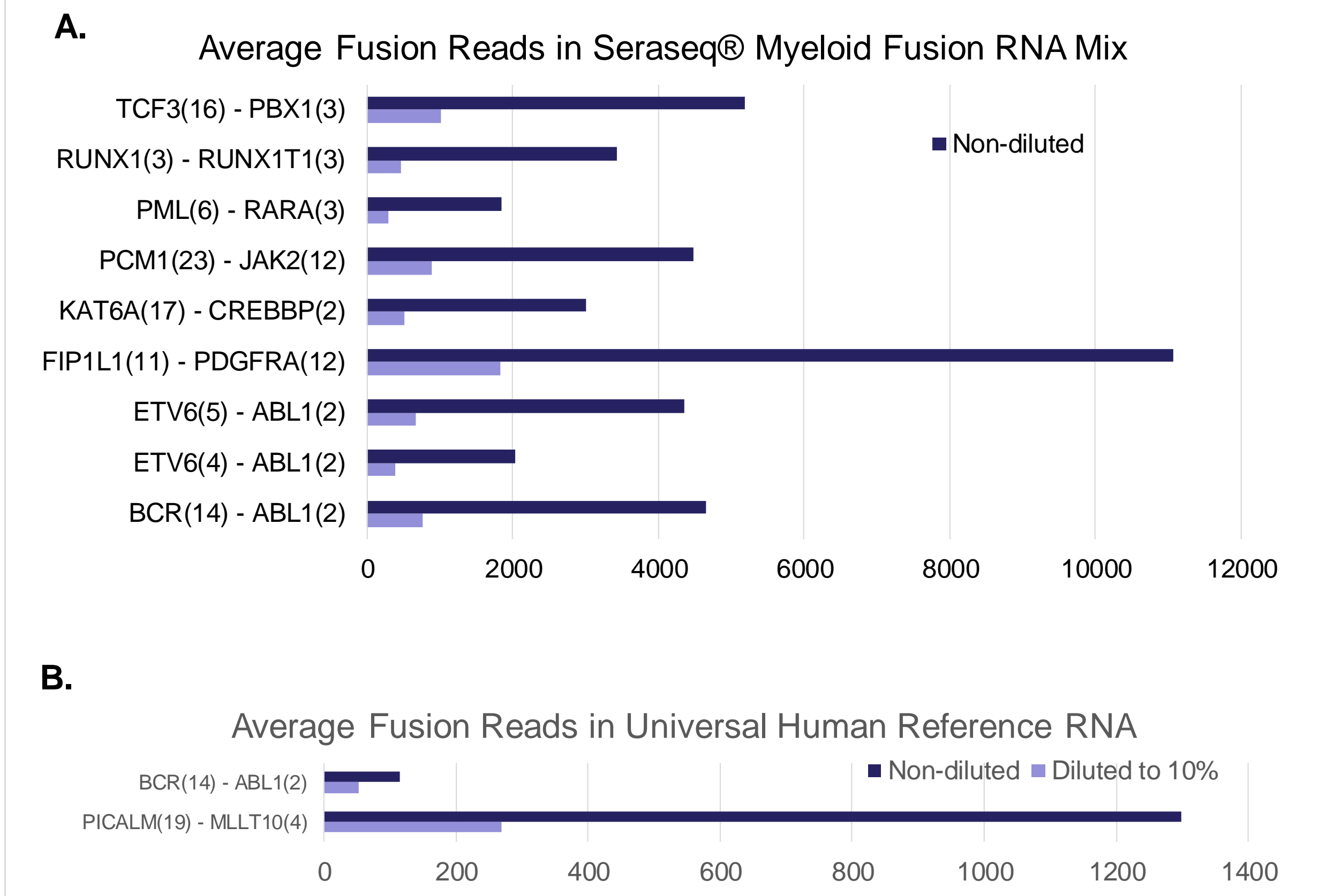
(B) Seraseq® Myeloid Mutation DNA Mix					
Gene	Expected VAF	AA Change	100% sample input	50% sample input	25% sample input
ASXL1	10%	p.Glu635ArgfsTer15	8.6%	4.1%	-
CALR	5%	p.Leu367ThrfsTer46	4.3%	1.9%	-
CEBPA	15%	p.Lys313dup	10.7%	4.3%	2.8%
CEBPA	15%	p.His24AlafsTer84	10.1%	3.6%	-
FLT3	10%	p.Lys602_Trp603msGlyAlaPheArgGluTyrGluTyrAspLeuLys	6.6%	3%	1%
FLT3	5%	p.Asn587_Asp600dup	3%	1.9%	0.6%
JAK2	10%	p.Asn542_Glu543del	7.7%	2.6%	-
NPM1	5%	p.Trp288CysfsTer12	2.5%	-	-
SRSF2	5%	p.Pro95_Arg102del	5.4%	2%	-

(B) Multiple replicates of Seraseq® Myeloid Mutation DNA Mix with different dilutions were tested, including 100% sample input (i.e. no dilution), 50% sample input (2-fold dilution), and 25% sample input (4-fold dilution). 9 InDels were detected, with 2 FLT3ITDs at 1% or lower.

(C) DNA reference standard					
Gene	Expected VAF	AA Change	100% dilution	75% dilution	50% dilution
BCOR	70%	p.Gln1208ThrfsTer8	73.5%	-	-
JAK2	5%	p.Phe537_Lys539delinsLeu	5.9%	2.7%	2.2%
NPM1	5%	p.Trp288CysfsTer12	3.6%	3.3%	-

(C) Multiple replicates of DNA reference standard with different dilutions were tested, including 100% dilution (i.e. no dilution), 75% sample input (1.33-fold dilution), and 50% sample input (2-fold dilution). 4 InDels were detected at AF as low as about 2%.

Fig 4. Fusion calling performance with Seraseq® Myeloid Fusion RNA Mix and Universal Human Reference RNA



Fusion detection performance was evaluated using Seraseq® Myeloid Fusion RNA mix and Universal Human Reference (UHR) RNA. Gene fusions in the control samples were sequenced and characterized using the Myeloid v2 Genexus research analysis workflows. The threshold for a positive fusion call was an absolute read count (x-axis) of ≥ 40 . Fig 4A. 10 replicates of Seraseq controls were sequenced at 100% sample input (i.e. no dilution) and 10% sample input (i.e. 10-fold dilution) and RNA QC passed runs were used for the analysis. The average fusion reads across replicates is shown in the plot. Observed sensitivity is 100% at both 100% and 10% sample inputs. Fig 4B. similar analysis was done for UHR RNA with 10 replicates. No false positive call above the read threshold (20) was observed in any of the controls. (Note: BCR-ABL is excluded for sensitivity calculation. The fusion is present at low levels and is not reliable but not a FP and not used in Sensitivity calculations.)

CONCLUSIONS

- A targeted NGS research assay was developed on the Genexus sequencing platform to support genomic profiling of myeloid malignancies.
- We demonstrated accurate reporting of multiple variant types, and the ability to obtain data within 24 hours from nucleic acid to report with minimal manual intervention.
- Our research suggests that automation of myeloid genomic profiling significantly reduces operating complexity and cost and lead to future advancements in oncology research.

REFERENCES

Additional information regarding OncoPrint Myeloid Assay on Genexus can be found at <https://www.oncoPrint.com/myeloid>.

ACKNOWLEDGEMENTS

We would like to thank the entire myeloid project team at Thermo Fisher Scientific.

DISCLAIMER

For research use only. Not for use in diagnostic procedures.

