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Development of an automated genomic profiling assay for myeloid malignancies research

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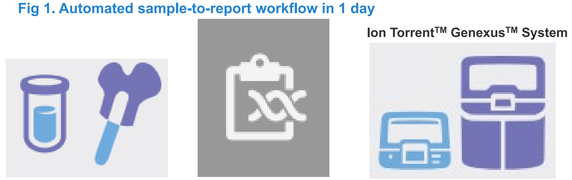
INTRODUCTION

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 Oncomine 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 Oncomine 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



✓ research samples containing whole blood, peripheral blood leukocytes (PBLs), and bone marrow

✓ Automated sample-to-report workflow, including library preparation, sequencing, variant calling, and reporting

✓ Annotated variant report with biomarkers linked to relevant evidence from public data sources

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 Oncomine Myeloid Assay GX

OVERVIEW

Table 1. Oncomine Myeloid Assay GX gene targets

DNA			RNA			
Hotspot only (28)		Full CDS (17)	Fusion driver (38)		Gene expression (5)	Expression control (5)
ABL1	SETBP1	ASXL1	KMT2A	KAT6A	BAALC	EIF2B1
ANKRD26	SF3B1	BCOR	BRAF	HMGA2	MECOM	FBXW2
BRAF	SMC1A	CALR	ABL1	KAT6B	MYC	PSMB2
CBL	SMC3	CEBPA	NUP98	NTRK2	SMC1A	PUM1
CSF3R	SRSF2	ETV6	RARA	NUP214	WT1	TRIM27
DDX41	U2AF1	EZH2	RUNX1	ABL2		
DNMT3A	WT1	IKZF1	PDGFRB	FGFR2		
FLT3		NF1	JAK2	MECOM		
GATA2		PHF6	PDGFRA	MKL1		
HRAS		PRPF8	FGFR1	MN1		
IDH1		RB1	ETV6	TFE3		
IDH2		RUNX1	MYH11	ALK		
JAK2		SH2B3	MLLT10	BCL2		
KIT		STAG2	TCF3	CCND1		
KRAS		TET2	NTRK3	EGFR		
MPL		TP53	ZNF384	FGFR3		
MYD88		ZRSR2	FLT3	FUS		
NPM1				MET		
NRAS				MYBL1		
PPM1D				NCOA2		
PTPN11				PAX5		

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 48 RNA genes.

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

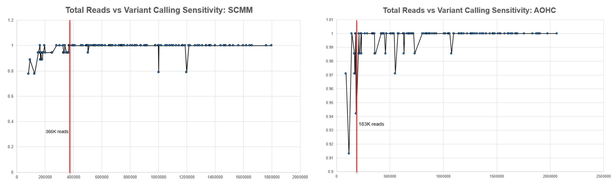
Hotspot control (70 variants expected)		
Run #	TP detected	Sensitivity
Rep1	69	98.6%
Rep2	70	100%
Rep3	69	98.6%
Rep4	69	98.6%
Rep5	70	100%
Rep6	70	100%
Rep7	70	100%
Rep8	70	100%
Rep9	70	100%
Rep10	69	98.6%
Overall	696/700	99.4%

Seraseq® Myeloid Mutation DNA Mix (20 expected)		
Run #	TP detected	Sensitivity
Rep1	20	100%
Rep2	19	95.0%
Rep3	19	95.0%
Rep4	19	95.0%
Rep5	19	95.0%
Rep6	19	95.0%
Rep7	19	95.0%
Rep8	19	95.0%
Rep9	19	95.0%
Rep10	19	95.0%
	191/200	95.5%

DNA reference standard (12 expected)		
Run #	TP detected	Sensitivity
Rep1	12	100%
Rep2	11	91.7%
Rep3	12	100%
Rep4	12	100%
Rep5	11	91.7%
Rep6	11	91.7%
Rep7	11	91.7%
Rep8	11	91.7%
Rep9	11	91.7%
Rep10	11	91.7%
	113/120	94.2%

The DNA variant calling performance was tested on 3 analytical controls, including AcroMetrix™ Oncology Hotspot Control (70 Oncomine variants expected), Seraseq® Myeloid Mutation DNA Mix (20 Oncomine variants expected), DNA reference standard (12 Oncomine variants expected). Oncomine Variants 5.16 filter was applied to filter for Oncomine 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.Leu676PhefsTer16	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_Trp603insGlyAlaPheArgGluTyrGluTyrAspLeuLys	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%	-

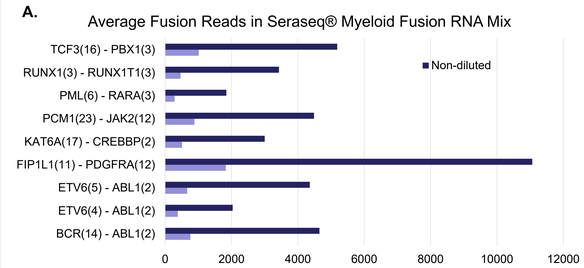
(B) Multiple replicates of Seraseq® Myeloid Mutation DNA Mix with different dilutions were tested, including 100% sample input (i.e. no dilution), 50% sample input (2-fold dilution), and 25% sample input (4-fold dilution). 9 InDels were detected, with 2 FLT3ITDs at 1% or lower.

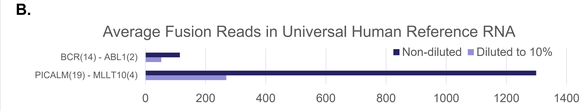
(C) DNA reference standard

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(R) 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 Oncomine Myeloid Assay on Genexus can be found at https://www.oncomine.com/myeloid.

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