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Fully automated sample-to-report NGS workflow for comprehensive genomic profiling of myeloid neoplasm research samples

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ABSTRACT

Oncomine™ Myeloid Assay GX v2 was developed to be utilized with the automated Ion Torrent Genexus System to profile genomic variants associated with myeloid malignancies. The software linked Genexus Purification System and Genexus Integrated Sequencer provides a quick and simplified automated NGS workflow with a one-day turnaround time from sample to variant reporting. Integrated DNA+RNA purification experiments with blood and bone marrow consistently met nucleic acid concentration requirements. Sequencing outcomes passed quality control metrics and robustly called genomic variants. Variant allele frequencies (AF) were detected down to 5% and were concordant with Gene Studio S5 sequencing data.

INTRODUCTION

Myeloid malignancies are associated with a broad and diverse set of genomic alterations, including SNVs, insertions, deletions and gene fusions[1]. Comprehensive characterization of genetic mutations in hematological disorders is complex and can take multiple days to complete.

Oncomine Myeloid Assay GX v2 paired with automated Genexus System offers an easy-to-use sample-to-report workflow processing of up to 8 (DNA+RNA) samples per day. DNA only and RNA only integrated runs are also possible. The Oncomine Myeloid Assay GX v2 provides comprehensive coverage of genetic targets relevant to myeloid disorder research such as Acute myeloid leukemia (AML), Myelodysplastic syndrome (MDS), Myeloproliferative neoplasms (MPN), Chronic myeloid leukemia (CML), Chronic myelomonocytic leukemia (CMML), and Juvenile myelomonocytic leukemia (JMML):

- DNA panel evaluates 1662 hotspots in 28 genes and fully covers 17 key genes;
- RNA panel includes 30 fusion driver genes and can detect 779 fusion isoforms

Table 1. Oncomine Myeloid Assay GX v2 Panel Targets

Hotspot genes (28)		Full genes (17)		Fusion Driver Genes (30)		Expression genes (5)	Expression control genes (5)
ABL1	*KRAS*	*ASXL1*	*PRPF8*	*ABL1*	*MET*	*BAALC*	*EIF2B1*
ANKRD26	*WT1*	*BCOR*	*RB1*	*ALK*	*MLLT10*	*MECOM*	*FBXW2*
BRAF	*MPL*	*CALR*	*RUNX1*	*BCL2*	*MLLT3*	*MYC*	*PSMB2*
CBL	*MYD88*	*CEBPA*	*SH2B3*	*BRAF*	*MYBL1*	*SMC1A*	*PUM1*
CSF3R	*NPM1*	*ETV6*	*STAG2*	*CCND1*	*MYH11*	*WT1*	*TRIM27*
DDX41	*NRAS*	*EZH2*	*TET2*	*CREBBP*	*NTRK3*		
DNMT3A	*PPM1D*	*IKZF1*	*TP53*	*EGFR*	*NUP214*		
FLT3	*PTPN11*	*NF1*	*ZRSR2*	*ETV6*	*NUP98*		
GATA2	*SETBP1*	*PHF6*		*FGFR1*	*PDGFRA*		
HRAS	*SF3B1*			*FGFR2*	*PDGFRB*		
IDH1	*SMC1A*			*FUS*	*RARA*		
IDH2	*SMC3*			*HMGA2*	*RBM15*		
JAK2	*SRSF2*			*JAK2*	*RUNX1*		
KIT	*U2AF1*			*KMT2A (MLL-PTD)*	*TCF3*		
				MECOM	*TFE3*		

MATERIALS AND METHODS

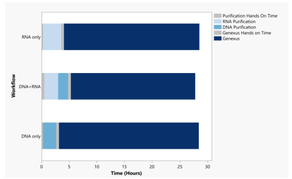
The Ion Torrent Genexus System is comprised of two software linked instruments: the Genexus Purification System and the Genexus Integrated Sequencer. The Genexus Purification System was used to isolate the DNA and RNA using the Multisample DNA Purification Kit and the Total RNA Purification Kit, respectively. Each run processed up to 12 blood or bone marrow samples from pre-characterized research myeloid samples representative of AML, MDS and healthy donor blood samples. Purification workflow included concentration quantification using Qubit instrument protocol. The Genexus Integrated Sequencer was used to dilute the extracted DNA to 1.1 ng/ul and extracted RNA to 0.95 ng/ul and sequence the samples in replicates with Oncomine Myeloid Assay GX v2.

Six DNA and RNA samples were sequenced per run per day along with commercially available analytical controls (Seraseq® Myeloid Mutation DNA Mix and Seraseq® Myeloid Fusion RNA Mix), and a No Template Control. Run reports were generated by the Genexus 6.6 Software analysis pipeline optimized to detect different variant types. Default Oncomine Variants (5.16) Filter Chain was used to generate Variant Reports. Bioanalyzer Pico RNA kit was used to assess RNA quality. Further analysis was conducted using JMP software.

RESULTS

Figure 1. Genexus Instruments

Figure 2. Turnaround Time



The integrated Genexus system is comprised of the Purification System (Fig 1.1) and the Genexus Integrated Sequencer (Fig 1.2). Genexus Software allows for linked run planning of combined DNA + RNA run with 6 samples + 1 positive and 1 negative control with a turnaround time from sample to report of 28 hours, allowing for processing of 8 samples per day (Fig 2). Standalone purification runs can also be carried out, accommodating up to 12 samples per run with a turn around time of 2.3 hours (DNA) or 3.3 (RNA).

Figure 3. DNA Purification

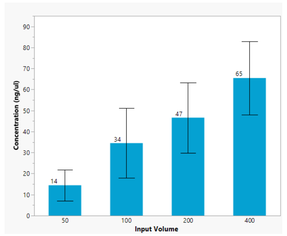
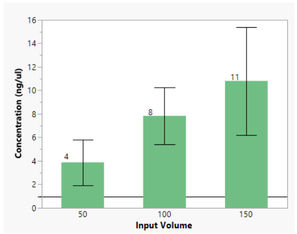


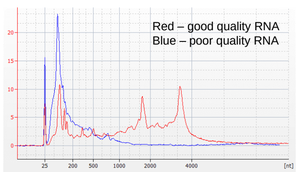
Figure 4. RNA Purification



DNA was isolated from whole blood using input volumes of 50, 100, 200 and 400ul across three available workflows dependent on input volume, using 31 unique samples with 134 replicates (Fig 3). All input volumes and workflows generated concentrations at or above the minimum requirement of 1.1 ng/ul for generating Myeloid libraries. Average DNA yield with 100ul blood input was 1672 ng.

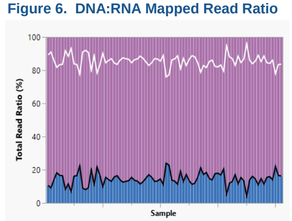
RNA purified from whole blood samples consistently produced the minimum required concentration of 0.95 ng/ul for the Myeloid RNA fusion panel (Fig 4). RNA biofluid workflow was used with 50, 100 and 150ul sample input for 34 unique samples with 213 replicates. Average RNA yield with 100ul blood input was 697 ng.

Figure 5. RNA Bioanalyzer Trace



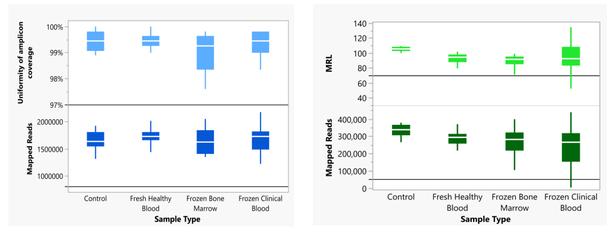
Bioanalyzer traces from RNA samples (Fig.5) reflected sample quality (good in red; poor in blue) and correlated with sequencing performance. Higher levels of RNA degradation were observed in samples undergoing a freeze/thaw cycle and characterized with high White Blood Cell Count.

Figure 6. DNA:RNA Mapped Read Ratio



The average ratio of total mapped reads was 85% DNA and 15% RNA (Fig 6) across healthy donor whole blood, AML whole blood and MDS bone marrow samples.

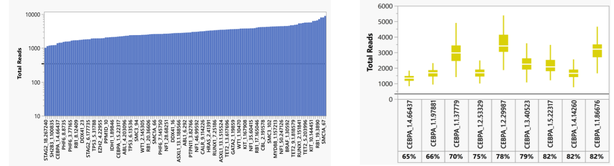
Figure 7. Sequencing Outcomes



Sample-to-result runs of blood and bone marrow samples consistently passed QC metrics for integrated purification and sequencing of DNA and RNA. DNA extracted from healthy donor blood, AML and MDS characterized blood and bone marrow aspirates were sequenced using DNA+RNA workflow. All 90 samples passed quality control metrics of 800,000 mapped reads and >97% uniformity of amplicon coverage (Fig 7A).

RNA isolates from healthy donor blood, AML donor blood and MDS bone marrow aspirates were sequenced using DNA+RNA workflow. 92/100 replicates passed quality control metrics of 50,000 reads and 70bp mean read length, MRL (Fig 7B). The failing samples were analyzed further and demonstrated low RNA quality including RIN numbers <2 and %DV200 <20%. RNA degradation was caused by abnormally high WBC counts in blood samples and freeze-thaw conditions due to sample storage requirements (Fig 5).

Figure 8. Amplicon Coverage

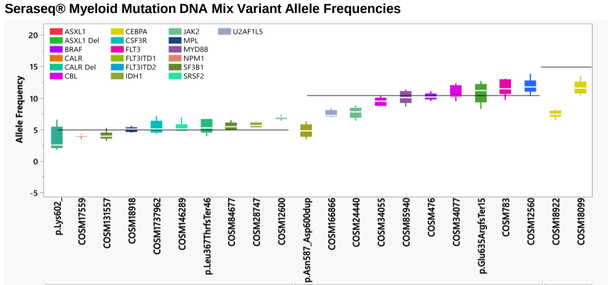


Oncomine Myeloid GX v2 Assay yielded robust amplicon coverage across multiple runs and sample types. Fig 8A shows Total Reads on Log10 scale for 140 amplicons with hotspots for DNA+RNA runs. All samples that satisfied pass/fail sequencing criteria had at least 99.5% of 1662 hotspots covered at 350x. 58 out of 61 samples had 350x hotspot coverage at 100%.

Oncomine Myeloid GX v2 Assay fully covers 17 genes including high GC content genes such as CEBPA. Fig 8B shows Total Reads for 9 CEBPA amplicons. All samples had 100% of CEBPA gene bases covered at 350x.

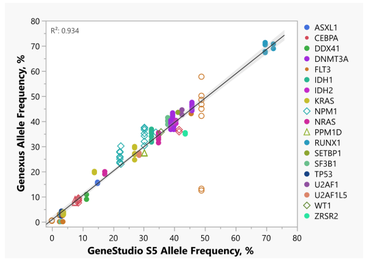
Figure 10. SNV/InDel and FLT3-ITD Detection in Control Sample

Seraseq® Myeloid Mutation DNA Mix Variant Allele Frequencies



Seraseq® Myeloid Mutation Mix includes 13 SNVs, 8 InDels, and 2 FLT3-ITDs variants. It was used to evaluate variant detection on Genexus 6.6SW with default Oncomine Variants 5.16 Filter Chain. Oncomine Myeloid Assay GX v2 reproducibly detected 22 out of 23 variants. COSM34210 ASXL1 p.G646fs*12 is G insertion on top of an 8G homopolymer and was not detected reproducibly. Excluding COSM34210, Sensitivity and Reproducibility were 100%. No False Positive variants were detected. Black lines indicate expected AF of variants.

Figure 11. SNV/InDel and FLT3-ITD Detection in Blood and Bone Marrow



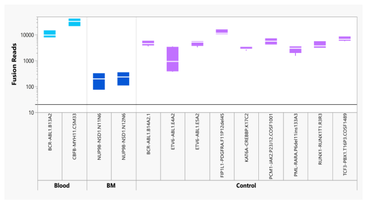
In addition to Genexus System, extracted blood and bone marrow samples were sequenced on GeneStudio S5 platform. Tight AF correlation (R^2 = 0.934) was seen between two NGS platforms for different variant types:

- ● SNV- filled circles;
- ◊ insertion – diamonds;
- Δ deletion – triangles;
- ○ FLT3-ITD – open circles.

Concordance between two NGS platforms was 99.4% for the variants down to 3% AF. It was calculated vs GeneStudio S5 data consensus as follows: **TP /(TP+FN+FP)**

True Positive (TP)
False Negative (FN)
False Positive (FP)

Figure 12. Fusion Detection



RNA fusion reads were evaluated using Seraseq® Myeloid Fusion RNA Mix Control. All nine expected fusions were detected across 7 replicates for 100% Sensitivity. At least 20 fusion reads are required to call fusion variants. No false positives fusions were observed.

Two AML blood and one MDS bone marrow (BM) samples detected a fusion variant in multiple replicates. The presence of Genexus detected fusions was confirmed on GeneStudio S5 platform.

CONCLUSIONS

The Genexus System integrates nucleic acid purification with sequencing and data analysis. Fully automated DNA+RNA specimen-to-report workflow with Oncomine Myeloid Assay GX v2 had turnaround time of 28 hours. DNA and RNA isolated from blood and bone marrow research samples on the Purification instrument met concentration input requirements for library preparation on Genexus. Sequencing QC criteria, including mapped reads, MRL and uniformity of amplicon coverage, passed for all DNA and 92% of RNA samples. DNA amplicon coverage was consistently above 350x including all hotspots locations and high GC amplicons such as CEPBA. SNVs, InDels and FLT3-ITDs were accurately called in Seraseq® Myeloid Mutation DNA Mix, AML blood and MDS bone marrow samples. We detected relevant myeloid cancer genetic variations (AF >5%) with high reproducibility and high concordance to the data generated on GeneStudio S5 NGS platform. RNA fusions from Seraseq Myeloid Fusion RNA Mix were correctly called with 100% sensitivity, and fusions were reproducibly reported in blood and bone marrow samples.

REFERENCES

1. Padmakumar, Devipriya, et al. "A concise review on the molecular genetics of acute myeloid leukemia." Leukemia Research 111 (2021): 106727.

ACKNOWLEDGEMENTS

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DISCLAIMER

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