Genexus System news

Issue 5: April 2021

In this issue we are bringing you more news

- Customer experiences with the Ion Torrent™ Oncomine™ Myeloid Research Assay GX on the Ion Torrent™ Genexus™ System
- How the Genexus System is taking center stage in epidemiological surveillance and detection of new mutations and strains of the SARS-CoV-2 virus
- Results of a workflow comparison study comparing hands-on time (HOT) for the Genexus System with the Illumina™ MiSeq™ System. The study demonstrated that the hands-off, set-up-and-go workflow of the Genexus System truly enables even lab personnel without any experience in NGS, and results in significant savings on hands-on time and resources

UAB study evaluates the Oncomine Myeloid Research Assay GX on the Genexus System

Performance of the Oncomine Myeloid Research Assay GX

Craig Mackinnon, MD, PhD, Director of Genomic Diagnostics and Bioinformatics and Professor of the Department of Pathology, and his team at the University of Alabama, Birmingham (UAB) conducted a study evaluating the performance of the Oncomine Myeloid Research Assay GX on the Genexus System with samples containing mutations in key genes associated with various myeloid research disorders. Difficult-to-detect targets such as *FLT3*, *CALR*, and *CEBPA* were included. Performance was assessed against the well-established Ion Torrent™ Oncomine™ Myeloid Research Assay on the Ion GeneStudio S5 System.

Molecular oncology research testing considerations

Dr. Mackinnon advises anyone interested in setting up a next-generation sequencing (NGS) panel in a molecular oncology research lab to consider a platform that can accommodate a wide range of specimens, including those that are small and/or low quantity. Sequencing challenges are compounded by the diversity of frequently co-existing complex variants underlying somatic changes present in many samples. Consequently, robust NGS assays capable of identifying single-nucleotide variants (SNV), small indels, and fusions in a single workflow are required.

(continued, next page)

“Quote of the month

The Genexus System has made our transition to rapid multigene testing in oncology a reality. Through the ‘Nexus’ initiative (a strategic partnership between the SJMC laboratory and key technology and pharma industry players), we are enabling the clinical research of potentially life-changing therapies of patients with advanced lung cancer in a timely and cost-effective manner.”

– Pathmanathan Rajadurai, MBBS, FRCPA, FRCPath, Senior Consultant Pathologist and Laboratory Director Medical Center Subang Jaya, and Professor of Anatomical Pathology at the University of Malaya Medical Center in Malaysia.
(on the photo on the left)

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Table 1. A range of commonly mutated genes in myeloid research malignancies.

Process	Gene	Disease	Variant
Signaling pathways	*FLT3*	AML, MDS, CMML, aCML	ITD (+12 to +300bp)
	JAK2	PV (95%) ET (50–60%)	V617F Exon 12 indels
	CALR	ET, PMF	+52 bp (type 1) +5 bp (type 2)
DNA methylation	*IDH1/2*	AML	SNV
	DNMT3A	AML	SNV, indel
Splicing	*SFRB1*	MDS	
Transcription factors	*CEBPA*	AML	Biallelic frameshifts, indels
	RUNX1	AML, MDS	SNV, indel
Tumor suppressor	*WT1*	AML	
Other	*NPM1*	AML, MDS	+4 bp

Table 2. Results show high concordance between the Genexus System and the GeneStudio S5 System.

Sample name	Expected variant	Called variants, Genexus System	Called variants, Ion GeneStudio S5 System
Sample 1	*CALR*	*CALR*	Detected
Sample 2	*FLT ITD*	*FLT3*	Detected
	NPM1	*NPM1*	Detected
	SFRB1	*SF3B1*	Detected
	DNMT3A	Not targeted*	Not detected in the Ion GeneStudio S5 System
	WT1	Not targeted*	Not detected in the Ion GeneStudio S5 System
Sample 3	*CEBPA*	*CEBPA*	Detected
	CEBPA	*CEBPA*	Detected
	IDH2	*IDH2*	Detected
	JACK2	*JACK2*	Detected
Sample 4	*IDH1*	*IDH1*	Detected
	DNMT3A	*DNMT3A*	Detected
	SRF2	*SRF2*	Detected
	RUNX1	*RUNX1*	Detected
	RUNX1 (2nd variant)	*RUNX1* (2nd variant)	Detected in the Ion GeneStudio S5 System
	JAK2 (low freq)	*JAK2* (3.5% AF) Detected in 1 of 2 samples in the Ion GeneStudio S5 and Genexus	

* The Oncomine myeloid assay doesn't provide full coverage for *DNMT3A* and *WT1* genes.

Summary of the evaluation

- The Oncomine Myeloid Research Assay GX achieved 100% concordance with Myeloid Research Assay on the GeneStudio S5 System
- The Genexus System demonstrated comparable sensitivity for gene detection for a wide variety of challenging variants, including difficult-to-detect targets such as *FLT3-ITD*, *CALR*, and *CEBPA*

Conclusion

In their quest to optimize their lab operations, UAB evaluated the Oncomine Myeloid Research Assay GX and the Genexus System. Dr. Mackinnon concluded that the Genexus System and its assays offer several key advantages over previous NGS platforms:

- Reduces overhead substantially due to lower reagent costs, less technical hands-on time, and reporting
- Delivers significantly faster turnaround times (2 days) with minimal hands-on time
- Detects challenging variants like indels and targets with GC-rich DNA
- Requires lower DNA and RNA input than other assays, permitting testing of a wider range of samples—an important consideration when sequencing FFPE samples

"The sensitivity of the Genexus System was superior for some of our targeted regions and we gained significant efficiency with turn around time, hands-on time, and reagent costs."

–Craig Mackinnon, MD, PhD, Director, Genomic Diagnostics and Bioinformatics–Professor, Department of Pathology, University of Alabama at Birmingham

View Dr. Mackinnon's presentation at
thermofisher.com/USCAP

Evaluating the Oncomine Myeloid Research Assay GX for detection of key variants

Dr. Yi Ding of Geisinger Medical Laboratories recently presented at the 2020 Association of Molecular Pathology annual meeting. During her talk, she described the value of NGS in hematopathology research and shared her experience utilizing the Oncomine Myeloid Research Assay GX and the Genexus System.

As the number of cancer-associated biomarkers has dramatically increased over the last few decades, molecular testing has become a critical component of precision oncology research. In recent years, NGS has played an increasingly important role in hematopathology, owing to its ability to rapidly and simultaneously interrogate multiple genomic aberrations from a single sample.

MPN screening algorithm

Myeloproliferative neoplasm (MPN) testing: single-gene testing vs. NGS

Molecular profiling of MPN samples can be particularly challenging due to their underlying genetic complexity. As such, MPN testing is a multifactorial process, which includes CBC value and molecular testing.

Recently, driver mutations have been identified in more than 90% of MPN samples, which has provided substantial insight into the pathogenesis of these diseases. Testing is generally conducted using the sequential model or with a multigene NGS panel.

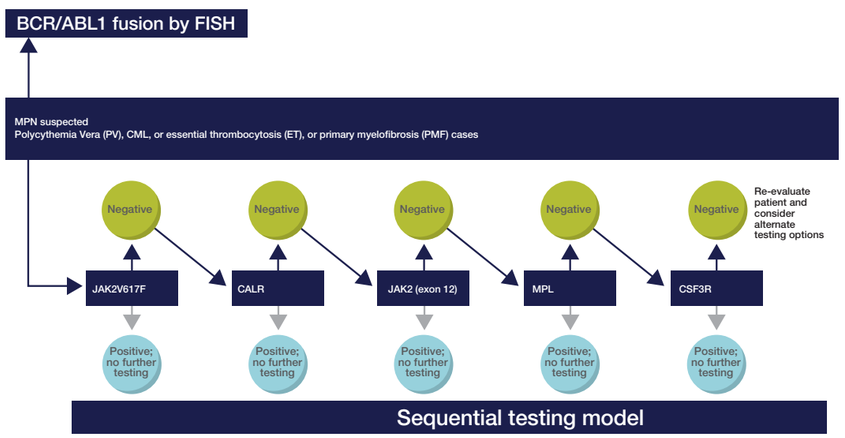


Figure 1. Sequential testing of MPN samples by Geisinger Medical Laboratories. For all samples, testing for the *BCR-ABL1* fusion and sequential gene mutation testing was conducted in parallel. *JAK2* v617F was tested first. If negative, *CALR* was tested next and so on. In Dr. Ding's lab, only the *JAK2* v617F was conducted in-house so there was usually a 4–6 week turnaround time for results. Since about 75% of the samples are negative, the majority of the patients had to go through the entire testing process, which can drive up costs and extend the time to answers.

"Most labs use STAT FISH or RT-PCR for PML-RARA detection because results are needed in <48 hours. Conventional NGS is not used, as it generally takes 4–5 days for results. With its automated processes and speed—from library preparation to variant interpretation in ~24 hours—the Genexus System has strong potential to create a new testing paradigm."

–Yi Ding, MD, PhD, System and Core Laboratory Director of Molecular Diagnostics, Geisinger Medical Laboratories

Evaluation of the Genexus System for potential detection of key MPN variants and translocations

Geisinger Medical Laboratories recently implemented a multigene NGS testing model that includes all the relevant genes and mutations. This approach enabled them to test for all relevant myeloproliferative neoplasm (MPN) mutations in a much more rapid and streamlined manner. As they are always looking to improve their capabilities, the lab recently evaluated the potential of the Oncomine Myeloid Research Assay GX for the detection of key variants and translocations associated with MPNs. With the Genexus System, the assay can yield results as fast as a single day.

Five samples were tested on each platform, including a variety of variants and allele frequencies—current in-house NGS test vs. the Oncomine Myeloid Research Assay GX. They included emerging MPN markers like *NRAS* and *TP53*. In addition, they assessed the potential of the assay to detect *FLT3*-ITD mutations and key fusions for myeloid disorder research.

Table 3. MPN research: Summary of current in-house NGS vs. the Oncomine Myeloid Research Assay GX on the Genexus System (n = 35).

Sample	Variant	Current NGS VAF (%)	Oncomine Myeloid Research assay on the Genexus System VAF (%)
GMC-1	CALR L367T46	55.3	51.8
GMC-3	CALR L367T46	16.7	11.6
GMC-16	CALR K385Nfs47	15	14.7
GMC-22	CALR K385Nfs47	8.8	7.7
GMC-5	CSF3R T618I	38.3	39.5
GMC-37	CSF3R T618I	34.6	34.3
GMC-87	CSF3R T618I	11.3	11.1
GMC-2	JAK2 V617F	55.1	20.2
GMC-8	JAK2 V617F	42.9	45.3
GMC-11	JAK2 V617F	34.7	33.2
GMC-13	JAK2 V617F	27.3	29.7
GMC-14	JAK2 V617F	9.7	11.8
GMC-19	JAK2 V617F	8.5	8
GMC-38	JAK2 V617F	8.3	8.5
GMC-56	JAK2 V617F	4.5	6.7
GMC-7	MPL R592*	50.9	49.6
GMC-55	MPL W515K	45.8	47.2
GMC-90	MPL S505N	9.1	10.1
GMC-93	MPL S505N	8.7	10.5

Oncomine Myeloid Research Assay GX evaluation results

- Achieved overall sensitivity of 97.1% for key MPN variants
- Demonstrated higher sensitivity than traditional cytogenetic methods for detecting *BCR-ABL1* fusions
- Attained accurate detection of common *FLT3*-ITD mutations
- Delivered good performance for other fusions

Gene	Cases	VAF (%)
JAK2	8/8	4.5–55.1
CALR	4/4	7.7–51.9
MPL	4/4	8.7–50.9
CSF3R	3/3	11.3–38.3
IDH1	4/4	4.6–46.8
IDH2	5/3	3–46.2
SH2B3	1/1	47.2
NRAS	4/5	4.4–45.8
TP53	3/3	8.4–75.9

Envisioning the future of molecular diagnostics

Given their comparable sensitivity and their inherent advantages over single-gene testing in terms of efficiency, speed, and cost, Dr. Ding expects that NGS for multigene panel tests will continue to grow in the future, creating a new paradigm in molecular testing.

View Dr. Ding's AMP workshop presentation at **thermofisher.com/us/en/home/global/forms/life-science/amp2020-workshop-genomic-profiling.html**

A workflow and hands-on time (HOT) comparison study with the Illumina MiSeq System

Study design

Four operators with varying levels of NGS experience were instructed to sequence the same number of samples on both the Illumina MiSeq System and the Genexus System.

Their experience ranged from <1 month to more than 5 years, and none of the operators had experience with the Genexus instrument.

For each operator, 30 minutes of training to go over the Genexus instrument was provided. MiSeq System training was also provided for users who were not familiar with MiSeq instrument operation.

Each operator then sequenced 8 samples on the MiSeq System and 8 samples on the Genexus System as illustrated in Figure 2.

A 2-pool targeted NGS assay was used for this study. Specifically, the Ion Torrent™ Oncomine™ Focus Assay and the equivalent panel for AmpliSeq™ for Illumina™ Focus Panel. An auditor measured and recorded user HOT for each step of the Ion Torrent™ Genexus™ Integrated Sequencer workflow and the Illumina MiSeq workflow.

Study 4 results

1. The Genexus System demonstrated a time savings of 33% in an 8-hour workday compared to the MiSeq System,

The ease of use and automation level minimize operator variability. Increased reproducibility provides greater confidence in successfully analyzing precious samples.

Based on Figure 2 data, the following two observations can be made:

- Regardless of experience level, the Genexus System requires an average 13 minutes of HOT to operate and start sequencing samples. Compare this to the almost 3 hours of HOT required for the MiSeq System. With the Genexus System, users realize a time savings of 33% in an 8-hour workday that can be used to perform other important tasks within the lab.
- The second thing to note is the variability in time on the MiSeq System between users and experience level, with approximately 1 hour 15 minutes separating the two extremes. This illustrates the amount of variability in operation and ultimately, the amount of variability that occurs within the sequencing runs, which can potentially affect results and reproducibility from sample to sample.

2. The Genexus System can save significant time for the operator as sequencing runs increase, which can lead to important savings on operator costs per run (Figures 3 and 4, next page).

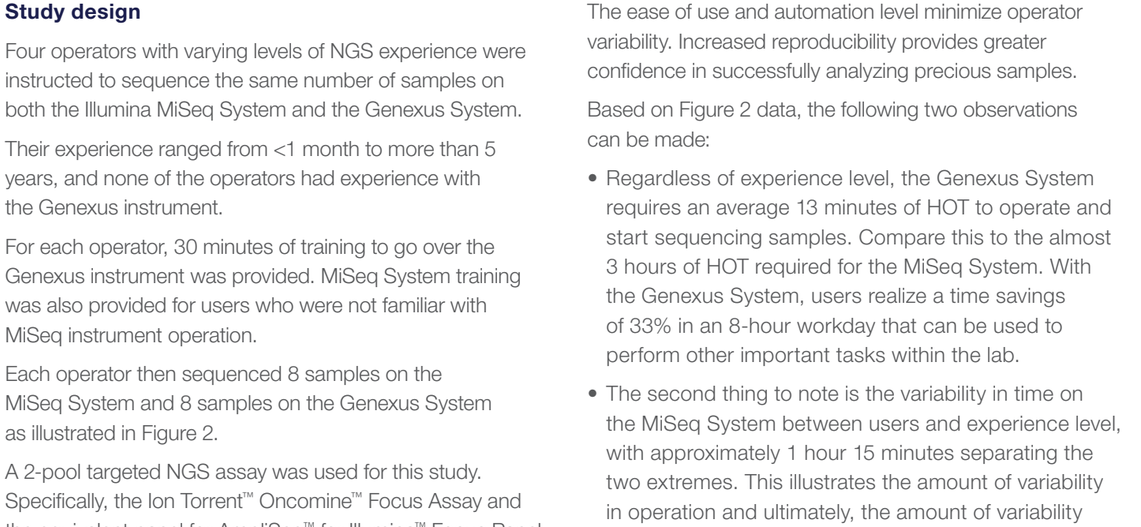
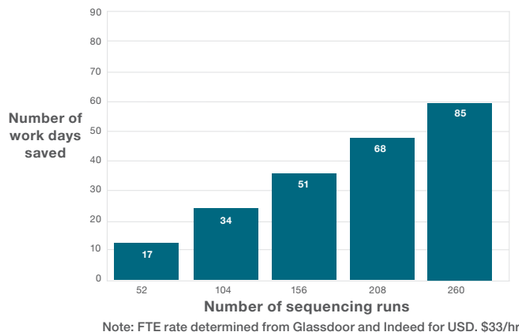


Figure 2. Hands-on time for each operator are shown in the plot below, with time for the MiSeq System shown in teal and the Genexus System in the dark purple.

Operator days saved by the Genexus System

The ease of use of the Genexus System saves a significant number of days for the operator as sequencing runs increase.



Note: FTE rate determined from Glassdoor and Indeed for USD. $33/hr

Figure 3. Ease of use with the Genexus System workflow saves a significant number of days as sample volume increases for a given period of time. Note: Calculations assume 1 sequencing run/day; 52 work days x 33% savings by the Genexus System = 17 work days saved (rounding down). Linear assumption of scales: 5 sequencing runs/working week and 52 weeks in a year = 260 sequencing runs.

Operator cost/run and savings by sequencing run

Operator cost savings by sample volume

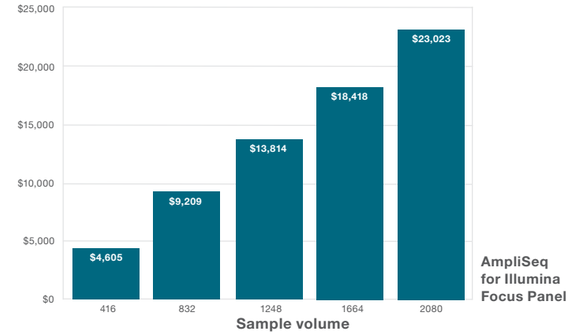


Figure 4. The difference in FTE costs/run are shown on the left. Using the same workflow as our comparison, you can see how the cost savings increase as the sample volume increases for a given period of time.

Setting up in a lockdown

How a remote support tool proved instrumental to a successful Genexus System installation under difficult circumstances

Circumstances surrounding the global crisis have made day-to-day operations, and certainly new equipment acquisition, challenging for labs across the globe. When researchers at one of the largest hospitals in Barcelona, Spain recently decided to enhance their next-generation sequencing (NGS) capabilities with a new Genexus System during a national lockdown, the Thermo Fisher Scientific team relied on help from the AR remote support tool to successfully install and set up the instrument.

A dedicated trio of skilled field service engineers were empowered to execute the installation by leveraging the support tool, which operated through mobile hotspots rather than the hospital's network, and provided the off-site engineer access to the on-site computers for installation operations.

Read how the Thermo Fisher team overcame many challenges and leveraged this tool to help the hospital install the Genexus System in 3 days under very difficult conditions.

Read the full story at
thermofisher.com/blog/behindthebench/setting-up-in-a-lockdown

Genexus System enabling SARS-CoV-2 genomic surveillance efforts

A school lab stepping up to help monitor local variants

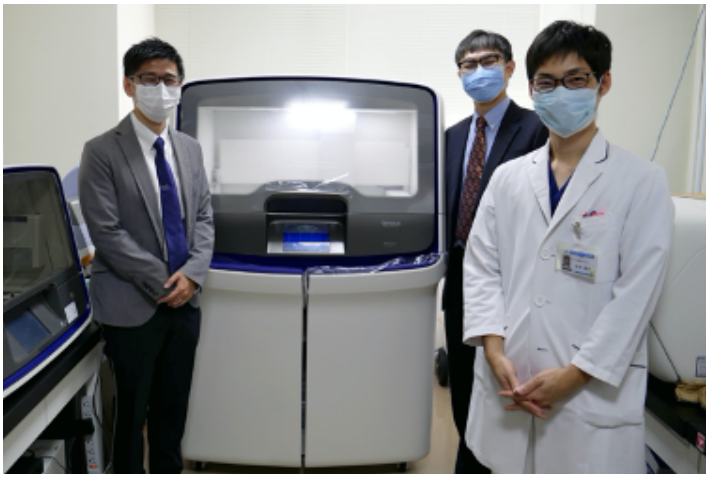
Drs. Dawn Richards and Elizabeth Forrester from the Baylor Esoteric and Molecular Laboratory at Baylor School are committed to improving regional SARS-CoV-2 genomic surveillance efforts. Affiliated with a private secondary school located in the city of Chattanooga, their lab has already been instrumental in providing qPCR-based testing for the population of Hamilton County, but after identifying the region's first case of the B.1.1.7 strain, the decision was made to expand capabilities through the use of NGS. Establishing an integrated workflow to continuously monitor prevalence and emergence of new variants in the population of the surrounding Chattanooga area was the goal, although providing that information to local health authorities in time to assist with critical decisions requires rapid turnaround time. Dr. Forrester, faculty member at the Baylor School and director of the Baylor Esoteric and Molecular Laboratory, was well aware of those challenges and immediately recognized the significance of the Genexus System in the context of regional surveillance. “If we were to outsource samples to a reference lab, it would take weeks to get results. With the Genexus System, we’ll be able to share sequencing results with public health officials in 24–48 hours.” Using the Ion AmpliSeq™ SARS-CoV-2 Research Panel on their Genexus System along with the integrated SARS-CoV-2 analysis workflow, the lab now has the ability to go from extracted sample to consensus sequence of the 30 kb viral genome in less than one day.

Dr. Dawn Richards, Laboratory Manager, and Dr. Elizabeth Forrester, Technical Director of the Baylor Esoteric and Molecular Laboratory, have transformed their high school teaching and research lab into a genomic surveillance center for the region.

The first P.1 lineage mutation in Japan was identified in February 2021 by the Ion AmpliSeq SARS-CoV-2 Research Panel on the Genexus System

Dr. Yosuke Hirotsu from Genome Analysis Center, Yamanashi Central Hospital, Japan was the first to report the P.1 SARS-CoV-2 variant, first identified in Brazil, was found in Japan. The sequencing analysis revealed that the SARS-CoV-2 strain of 20J/501Y.V3 (P.1 lineage) had 37 mutations including 22 missense, 10 synonymous, 3 intergenic, 1 frameshift, and 1 in-frameshift mutations. In spike protein, they observed 12 missense mutations (L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, and V1176F). These mutations were perfectly matched with the mutations in P.1 lineage previously discovered in Brazil. In RBD of spike protein, three mutations (K417T, E484K, and N501Y) were identified. These results suggested they identified the emerging strain related to 20J/501Y.V3 (P.1 lineage) in Japan.

Their experience with the Genexus System was very good. To date, they have analyzed 135 samples and received results from 133. The two cases had very low viral load. They appreciate the ease of use and fast TAT.



Dr. Yosuke Hirotsu, and Kazuo Sakai and Ryota Tanaka from Thermo Fisher Scientific.

* Specimen-to-report workflow will be available after the Ion Torrent™ Genexus™ Purification System and integrated reporting capabilities are added in 2021.

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