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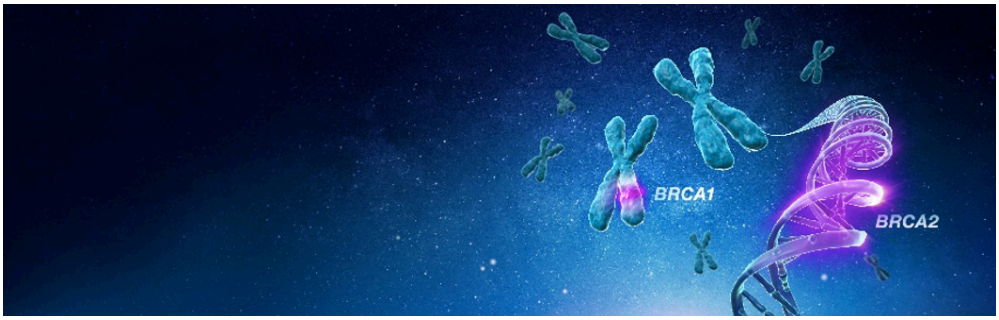
Genexus System News

Issue 8: April 2022



Genexus oncology research assays expansion continues

Introducing the Ion Torrent™ Oncomine™ BRCA Assay GX, a highly automated *BRCA* sequencing solution deployed on the revolutionary Ion Torrent™ Genexus™ System. Labs can now detect *BRCA* somatic and germline mutations from formalin-fixed, paraffin-embedded (FFPE) tissue and whole blood in a specimen-to-report workflow in as little as 24 hours, with only two user touchpoints and 20 minutes of hands-on time (HoT).

Key benefits of the Oncomine BRCA Assay GX include:

- Based on proven Ion AmpliSeq™ technology
- Requires only 20 ng of DNA input
- 100% exonic coverage with large intronic flanking regions
- Detects large genomic rearrangement, such as large insertions and deletions (indels) and exon-level duplication and deletion, without the need to employ multiple technologies

Learn more about the Oncomine BRCA Assay GX here.

Table 1. Internal data showed superior accuracy in detecting somatic and germline variants and high consistency independent of the workflow as well as platform. Positive predictive value (PPV) = true positives/total number of positives. Sensitivity = true positives/(true positives + false positives).

Control sample variants	Platform	Library	SNV		Indel	
			Sensitivity (%)	PPV (%)	Sensitivity (%)	PPV (%)
5% allele frequency	GeneStudio S5 System	Manual	100	98	98	92
	Genexus System	Ion Chef Instrument	100	92	99	99
		Genexus System	100	100	98	100
50%, 100% allele frequency	GeneStudio S5 System	Manual	100	100	100	100
		Ion Chef Instrument	100	100	100	100
	Genexus System	Genexus System	100	100	94	100

Quote of the month

"The Genexus System with its automated processing offers an advantage over other NGS systems by reducing turnaround times as well as operating costs."

– Luis Mendoza, BSc, NGS Lab Coordinator of LABOPAT

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In this section of Genexus System News, we are going to share more stories and experiences from labs around the world

Genexus System and Oncomine Precision Assay implementation in LABOPAT in Mexico

The Ion Torrent™ Genexus™ Integrated Sequencer with its automated processing, which helps to reduce response times and operating costs, is favored by our lab as a clinical research tool.

The LABOPAT sequencing laboratory implemented the Oncomine™ Precision Assay as the first Next-generation sequencing (NGS)-based test using the Genexus System equipment. So far, 60 samples have been processed as part of analytical verification.

The process lasted approximately seven months. It was designed accordingly to the guidelines of the College of American Pathologists (CAP), the Association of Molecular Pathology (AMP), and the requirements of the New York State Department of Health (NYS).

Synthetic controls with allelic frequency (AF) variation (10, 5, and 2.5%), copy number variation (CNV), and fusions and cell lines were sequenced. Nineteen samples from cancer patients with previous molecular biology (NGS, PCR, FISH) or immunohistochemistry (IHC) results were also included. Specificity, sensitivity, reproducibility, and analytical accuracy parameters for base and variant calling were evaluated (Table 2).

The short response times and higher reliability obtained from workflow automation has been appreciated by our collaborators.

Lastly, as direct users of the Genexus System and considering NGS is still an expensive technology for the vast majority of people in Latin American countries (including Mexico), we have found it to be a sequencing platform that not only has several operational advantages but also favors a large number of samples. Therefore, the Genexus System represents an advantage over others in the precision medicine research advancements.

Table 2. Specificity, sensitivity, reproducibility, and analytical accuracy parameters for base and variant calling.

Component	SNV	Indel	CNV	Fusion
Cutoff recommendation based on composite AV dataset	≥3% AF at 100x depth	≥3% AF at 100x depth	≥6 copies	≥20 reads (>7 molecules for MET exon 14 skipping)
Analytical accuracy of basecalling	99.46%			
Analytical specificity	99.4%	100%	100%	100%
Analytical sensitivity	100% above 5% allelic fraction	100% above 5% allelic fraction	100% above 6 copies	100% above 1000 copies
Variant calling accuracy	95%	100%	100%	100%
Reproducibility	100%	100%	100%	100%

As we are all hoping that we are nearing the end of the virus crisis, the Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay on the Genexus Sequencer has been instrumental to surveillance around the world.

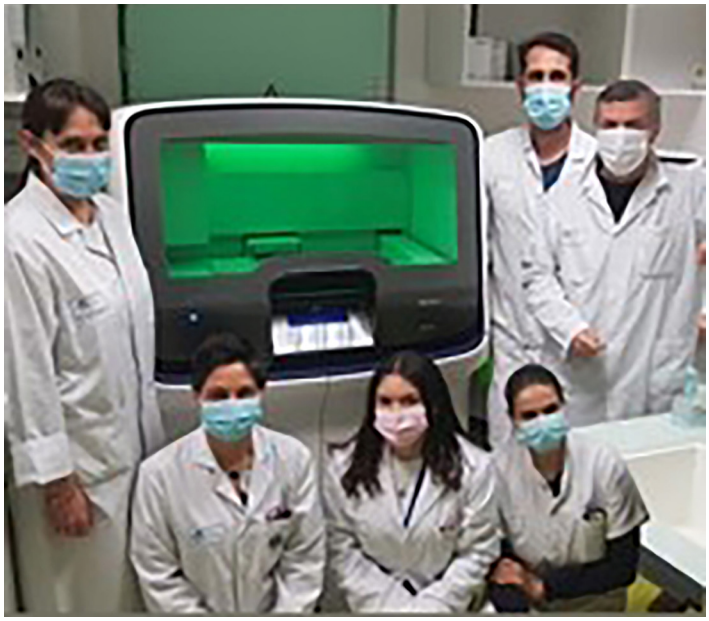
Among the first to detect the Omicron variant last year were clinical virologist Dr. Allison Glass and her team at Lancet Laboratories in Gauteng, South Africa. According to Dr. Glass, PCR results hinted at the virus' potential evolution. "We first detected the possibility of a new variant in early November after noticing an S-gene dropout on our SARS-CoV-2 detection using Thermo Fisher Scientific PCR testing kits," said Dr. Glass. "By then sequencing these samples in-house with the Genexus Integrated Sequencer, we were able to analyze and identify the specific virus mutations in less than 24 hours and alert the South African national surveillance program for confirmation and swift action. This speed of testing and sequencing is critical to help inform public health decisions and keep our populations safe through this new variant and those that are yet to emerge."

Also in the **Czech Republic,** Dr. Iva Dolinová from Liberec Regional Hospital has been using this solution for surveillance. In December 2021, she had detected the first case of the Omicron variant in the Czech Republic. "We have been able to confirm the new Omicron strain in a female patient within less than 24 hours using the very sensitive Ion AmpliSeq SARS-CoV-2 Insight Research Assay," said Dr. Dolinová, head of the Department of Genetics and Molecular Diagnostics. "This would have not been possible without the fast, automated Genexus Integrated Sequencer, our committed team, and our collaboration with Thermo Fisher Scientific."

In **France,** Dr. Paul Hofman of the Laboratory of Clinical and Experimental Pathology at the Centre Hospitalier Universitaire of Nice, France, demonstrated the Genexus Integrated Sequencer as an all-in-one NGS solution designed for frequent, low-volume use, making it ideal for SARS-CoV-2 genomic surveillance in smaller labs around the world.

Epidemiological surveillance at the scale needed to stay ahead of SARS-CoV-2 requires being able to sequence samples from patients with low viral loads, including asymptomatic patients, since these vastly outnumber hospitalizations. If only hospital-level samples are sequenced, this provides a skewed reading of the viral strains circulating in the larger population. The Genexus System is not only sensitive enough to generate robust sequences from samples with low viral loads, it can do so from multiple sample types, including nasopharyngeal swabs and saliva. For example, Dr. Hofman's team found excellent concordance in SARS-CoV-2 sequencing results for matched nasopharyngeal swabs and saliva samples, supporting more widespread use of saliva samples as a convenient collection method for SARS-CoV-2 surveillance.[1]

1. Hofman P, O Bordone, E Chamorey, et al. (2022) Setting-Up a Rapid SARS-CoV-2 Genome Assessment by Next-Generation Sequencing in an Academic Hospital Center (LPCE, Louis Pasteur Hospital, Nice, France). Front. Med. 8.



Watch a webinar by Dr. Paul Hofman on **Pivoting from cancer to COVID-19 in a global pandemic**

Read more about the Genexus System at **oncomine.com/genexus** and **thermofisher.com/genexus**

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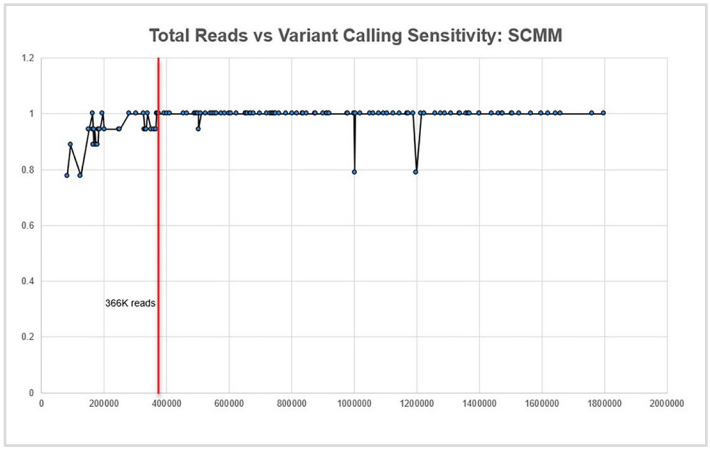
In the past, NGS technology has been found in well-staffed and well-resourced labs in academic centers, where most genomics research takes place. We are very proud to see how the Genexus System has been turning this pattern on its head all over the world. Another example is the **Limpopo province of South Africa** and its advanced genomic testing facility at Limpopo's Ndlovu Care Group, which was founded in 1994 by Dutch researcher Dr. Hugo Tempelman.

The laboratory performs upward of 2,000 PCR tests per day for Limpopo and its neighboring provinces, and it became central to the region's strategy during the emergence of SARS-CoV-2 as the world's preeminent infectious disease concern. They built up a database of 20,000 positive SARS-CoV-2 samples collected throughout the region, which are now used for genomic research with their Genexus sequencer, expanding Africa's genomic surveillance network with fast, powerful sequencing technology to discover new SARS-CoV-2 variants and help drive the development of public health strategies that may save lives.

Thanks to these world-class efforts, South Africa has been at the forefront of detecting new SARS-CoV-2 variants of concern, including Omicron, and will likely remain so for the foreseeable future. Bringing NGS to rural areas has a global impact on SARS-CoV-2 surveillance, and it has never been more possible.

Comprehensive data from our R&D team's poster presentation, "Development of an automated genomic profiling assay for myeloid malignancies research," given at AMP 2021.

In the results, Jiajie Huang et al. concluded that the sequencing metrics of the assay demonstrated high (>98%) uniformity and consistent read depth (>2,000x). 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 turnaround time (TAT) for 8 DNA and 8 RNA samples was 22.5 hours and the total HoT was 20 minutes.



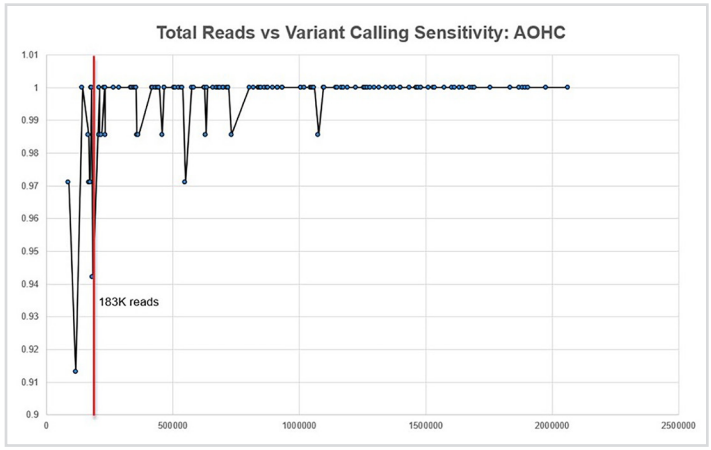


Figure 1. Sensitivity vs read depth in DNA variant calling. Sequencing results with control samples were downsampled to vary the number of mapped reads. With high uniformity of amplification 200K mapped reads in AOHC and 400K reads in SeraCare™ Seraseq™ Myeloid Mutation Mix were required to support 95% sensitivity.

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

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%	2.46%	3.09%
RB1	5–15%	p.Leu676PhefsTer16	7.08%	7.44%	6.71%	7.05%	6.92%	2.3%	3.13%

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 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.

Seraseq Myeloid Mutation DNA Mix					
Genes	**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_Trp603insGlyAla PheArgGluTyrGluTyrAspLeuLys	6.6%	3.0%	1.0%
FLT3	5%	p.Asn587_Asp600dup	3.0%	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%	2.0%	-

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). Nine indels were detected, with 2 *FLT3*-ITDs at 1% or lower.

DNA reference standard					
Genes	**Expected VAF**	**AA change**	**100% dilution**	**75% dilution**	**50% dilution**
BCOR	70%	p.Gin1208ThrfsTer8	73.5%	–	–
JAK2	5%	p.Phe537_Lys539delinsLeu	5.9%	2.7%	2.2%
NPM1	5%	p.Trp288CysfsTer12	3.6%	3.3%	–

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). Four indels were detected at AF as low as about 2%.

View the entire poster **click here.**

What has been going on?

OncomineWorld 2022
A virtual NGS education meeting

Join us on demand for the 4th edition of **Oncomine World 2022**. You will hear from more new labs using the Genexus System from all over the world, and take part in virtual Genexus System and software demonstrations.

Register now

And finally, on March 30, 2022, we have launched a webinar announcing the Ion Torrent™ Genexus™ Integrated Sequencer Dx.* It will be available soon in all countries recognizing CE-IVD designations. View on demand to hear from our leadership about this first step in bringing fast and easy NGS to clinical labs.

Register now

*** The Genexus Integrated Sequencer Dx is for *In Vitro* Diagnostic use in the EU. Not for diagnostic use in North America.**

Read more about the Genexus System at
oncomine.com/genexus and **thermofisher.com/genexus**

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