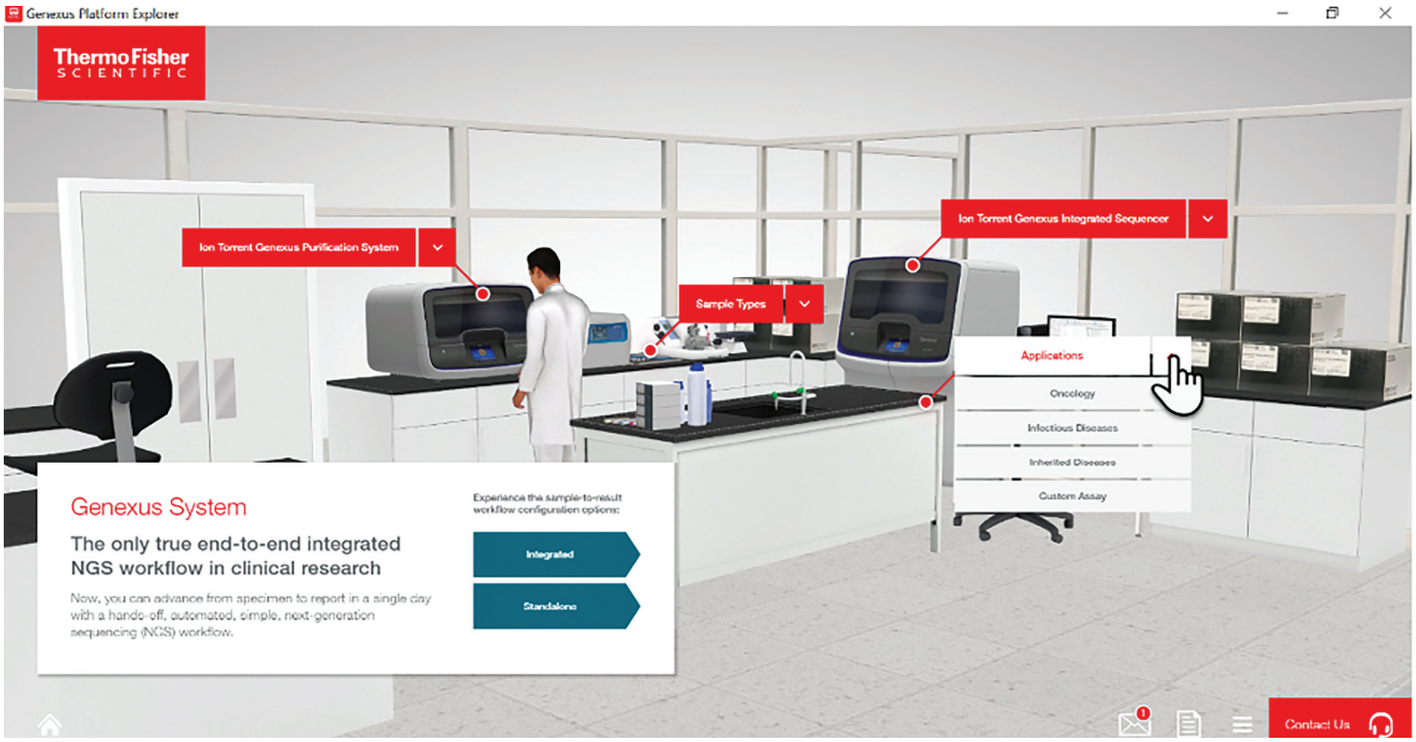
Genexus System News

Issue 9: August 2022



New Virtual Demo of Genexus System

Thinking about how the Genexus System works and how it could fit into your lab? In our virtual laboratory environment powered by augmented reality (AR) technology, you can see:

- How the Ion Torrent™ Genexus™ Integrated Sequencer and Genexus™ Purification System fit into your space
- Each step of the NGS workflow, including purification, sequencing, and analysis
- Sample types and applications supported by the Genexus System

Try the virtual demo here

Interview with Dr. Lara Navarro, Head Biologist in the Anatomical Pathology Department of the General University Hospital of Valencia, Spain

Dr. Navarro's lab, like many others over the last decade, has brought in new approaches to cancer sample testing. Not only do they use conventional methods such as immunohistochemistry and immunofluorescence, but they have also implemented complementary molecular biology techniques for genomic profiling. We sat down over Zoom with Lara to discuss her lab's experience implementing in-house next-generation sequencing (NGS).

Can you tell us about biomarker testing in your lab?

We have 12 to 15 samples each week (so about 50 cases per month), mainly *EGFR, ALK, ROS, RET, MET,* and Her-2. Before we brought in NGS, our biomarker testing was done gene by gene; we mainly used rtPCR, pyrosequencing, and – of course – Sanger sequencing.

Why did you decide to implement NGS – and were there any worries in your mind?

The main reason for the change was that we were increasingly required to test for multiple biomarkers with the smallest amount of sample possible. We needed a new technique that would allow us to conduct many simultaneous tests. Our main worry was whether the workflow was going to be realistically implementable in our laboratory. Would it be too time-consuming? Would it require too many resources? We were very surprised (in a positive way!) by the Genexus System. It was so straightforward and so fast that, almost as soon as it was installed, we started using it for testing. The workflow is one of the simplest we have here in the laboratory. All the required tasks (such as adding the reagents) are easy to perform and the software checks after the fact to help to ensure that no errors have been made. The consolidation of the workflow from sample to report, the speed, and the automation all make the system easy to implement and a great fit for our laboratory.

How does the Genexus System reduce hands-on time in your laboratory?

The process is practically fully automated and requires only about 20 minutes of hands-on time altogether – from nucleic acid extraction to final result and report. I think techniques such as fluorescence in situ hybridization or immunohistochemistry require the same, or perhaps even less, hands-on time – but analyzing genes using Sanger sequencing requires significantly more hands-on time and can take days to yield a result. For laboratories like ours, speed and efficiency are vital. We receive samples every day and need to process them fast, so we could not have implemented previous incarnations of NGS that required a lot of hands-on time and take several days to produce a result.

What is your turnaround time for results?

It takes us a maximum of two working days from getting a sample to returning a report. To be more precise, it takes us two working mornings, because we don't work on this type of testing later in the day. If a biomarker test is requested through the department information system on Monday morning, we have the report ready by Wednesday.

What panel do you use – and for what samples?

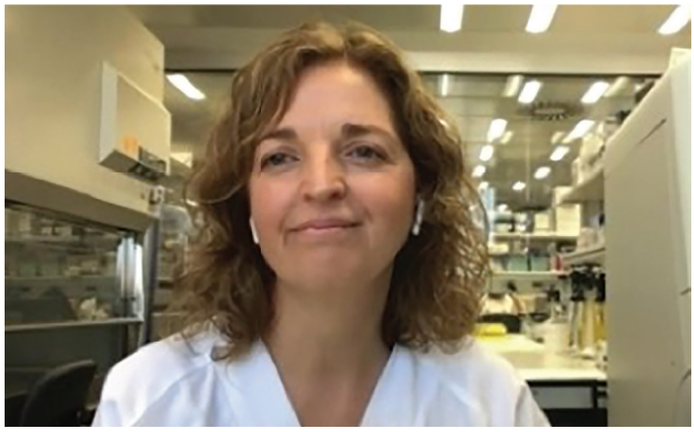
We use the Oncomine Precision Assay 50-gene panel, which covers all the biomarkers we need to test. Most of the samples we test are lung cancers, which are notoriously small because most are taken via bronchoscopy. Most of the time, we have only one segment available, which needs to stretch to all the necessary testing. That is why we value the Oncomine Precision Assay so much; it is an efficient way to use a small sample. We try to use samples with at least 20 percent tumor cells, but we do occasionally have to go as low as 5 percent – and, even when that happens, 98 percent of samples yield a conclusive result. Although lung cancer constitutes the majority of our samples, we also use the assay for colon cancers, melanomas, gliomas, some breast cancer samples, and some thyroid cancers.

Can you describe the bioinformatics software user experience?

The system employs one user-friendly software interface for the whole workflow, which makes our work very easy. We were also able to create a traceability system so that, at any time, we can see what samples have been sequenced, when they were sequenced, and what else our laboratory did with the sample – not to mention downloading the report.

How would you summarize your experience with NGS so far?

Honestly, based on what we have seen in the past with other platforms, we didn't think it would be possible to implement NGS in our lab. We were pleasantly surprised! With the Genexus System, the level of automation is such that very little hands-on work is needed, and our turnaround time is very short. The implementation process went smoothly and, now that we are fully up and running, we no longer experience sample accumulation and work overload. It has changed – very much for the better – the way we test for oncology biomarkers in our lab.



Dr. Lara Navarro

In this section we feature laboratory experiences with the Oncomine Precision Assay on the Ion Torrent Genexus System, as presented at Oncomine World and Ion World 2022

Hamilton Regional Laboratory Medicine Program; Experience with multi-variant NGS panel validation, presented by Daria Grafodatskaya, PhD, FCCMG.

In the laboratory of Dr. Daria Grafodatskaya, they performed an evaluation of performance for the Oncomine Precision Assay on 51 specimens including the following variants: 23 single nucleotide variants (SNV), 13 insertion-deletions (indels), and 17 complex indels characterized by their current methodology Ion AmpliSeq™ Cancer Hotspot Panel v2 on Ion S5. They established accuracy, reproducibility, lower limit of detection and lower limit of input. For fusion detection evaluation they also used reference standards HD874 (Horizon, 3 fusions), Seraseq® Fusion RNA (Seracare 18 fusions, 15 covered on the assay) and NTRK Fusion RNA Reference Material (15 Fusions). A total of 28 unique fusion events are present across three standards, and all were detected. In addition to the evaluation results, Dr. Grafodatskaja presented interesting cases demonstrating the advantages of using the 50 gene NGS panel versus smaller panels and single gene technologies.

Implementation of next-generation sequencing in solid tumors using the Genexus platform in Pathology Unit, San Luigi Hospital in Orbassano, Turin, Italy presented by Prof. Marco Volante, MD.

Prof. Volante walked through his laboratory experience with a previous Ion Torrent NGS platform and Oncomine assays, comparing them with the Genexus platform and Oncomine Precision Assay. In his summary he stated that:

- Optimization of NGS platforms should be tailored based on characteristics of users and sample type.
- Given the increasing number of tests to be performed, the technological equipment and workflows should consider both the expected sample load and the available resources.
- In our real life, the acquisition of Genexus platform increased optimization of human resources, with special reference reducing the time commitment, the complexity of the procedure and the risk of possible errors.
- In addition, the reduced turnaround time potentially allows a higher number of samples to be analyzed in the weekly workflow, with no parallel increase of the mean turnaround time.

Figure 1. Establishment of lower limit of detection (LOD) from Hamilton laboratory

Dilution	Read counts		Mol cov mutant	
	Expected	Observed	Expected	Observed
EML4(13)-*ALK*(20): 1:10 R1	67	289	5	9
EML4(13)-*ALK*(20): 1:10 R2	67	57	5	1
EML4(13)-*ALK*(20): 1:30	22	17	1.7	1
EZR(10)-*ROS1*(34): 1:10	149	103	18	10
EML4(13)-*ALK*(20): 1:30	47	303	11	17
EZR(10)-*ROS1*(34): 1:30	86	62	16	11

Figure 2. Comparison of some parameters of GeneStudio and Genexus workflows in Turin laboratory

Pathology Unit - San Luigi Hospital	GeneStudio S5 (~1800 samples)	Genexus (450 samples)
Pathologist time commitment	2 days/week	2 days/week
Molecular biologist time commitment	3 days/week	3 days/week
Lab technician time commitment	4.5 days/week	3 days/week
Maximum sample load/week	32	64 (2 possible runs of sequencing)
Time for pure technical procedure (from end of nucleic acid quality check to report)	3 days	1-2 days (depending on sample load)

Department of Molecular Diagnostics at the Holycross Cancer Centre in Kielce experience with Genexus System and evaluation of the Oncomine™ BRCA Assay GX, presented by Artur Kowalik, PhD, head of the department.

Dr. Kowalik presented the laboratory experience with the Oncomine BRCA Assay on GeneStudio S5 system and the concordance with the new version of the assay on Genexus System. He noted that whilst the concordance is high, the Genexus workflow is much faster, producing results in as little as in 14 hours.

Figure 3: Concordance of results generated by the Oncomine BRCA assay on Genestudio S5 and Genexus System in Kielce laboratory

Disease Category	Sample	Con ng/ul	Mutations (nt)	Mutations (aa)	Gene	Exon	Classification	S5 (AF%)	GX (AF%)
Ovarian Cancer	FFPE	35	c.4440T>G	p.Tyr1480Ter	*BRCA2*	11	5	53	51
Ovarian Cancer	FFPE	30	c.4965C>G	p.Tyr1655Ter	*BRCA2*	11	5	40	34
Ovarian Cancer	FFPE	42	c.5226dupC	p.(Gln1756Profs*73)	*BRCA1*	19	5	53	49
Ovarian Cancer	FFPE	78	c.8420C>A	p.(Ser2807Ter)	*BRCA2*	19	5	13	13
Ovarian Cancer	FFPE	177	c.9227G>A	p.(Gly3076Glu)	*BRCA2*	24	5	64	64
Ovarian Cancer	FFPE	99.7	c.181T>G	p.(Cys61Gly)	*BRCA1*	80	5	40	79.3
Ovarian Cancer	FFPE	22.5	c.181T>G	p.(Cys61Gly)	*BRCA1*	82	5	40	81
Ovarian Cancer	FFPE	65.8	c.5402del	p.(Gly1801AlafsTer33)	*BRCA1*	77	5	21	76
Ovarian Cancer	FFPE	2.9	c.5266dup	p.(Gln1756Profs*73)	*BRCA1*	71	5	19	69.4
Ovarian Cancer	FFPE	28.8	c.3974_39758 hsGCTT	p.(Ala1326LeufsTer5)	*BRCA2*	70	5	11	67.8
Ovarian Cancer	FFPE	182	c.5251C>T	p.(Arg1751Ter)	*BRCA1*	87	5	19	88
Ovarian Cancer	FFPE	125	c.2158G>T c.818C>A c.6385G>T c.6685G>T	p.(Glu720Ter) p.(Ser273Ter) p.(Glu2129Ter) p.(Glu2229Ter)	*BRCA1* *BRCA2* *BRCA2* *BRCA2*	10 10 11 11	5	40 31 39 35	37 37 33 33
Ovarian Cancer	FFPE	2.5	c.9371A>T	p.(Asn3124Ile)	*BRCA2*	25	5	57	62.9
Ovarian Cancer	FFPE	23.9	c.8378G>T	p.(Gly2793Val)	*BRCA2*	19	4	19	19.7
Ovarian Cancer	Blood	19.8	c.191G>A	p.(Cys64Tyr)	*BRCA1*	4	5	48	47.9
Pancreatic Cancer	Blood	25.8	c.7007G>A	p.Arg2336His	*BRCA2*	13	5	56	51.8

Figure 4: Examples of coverage of the Oncomine BRCA Assay GX in Kielce laboratory

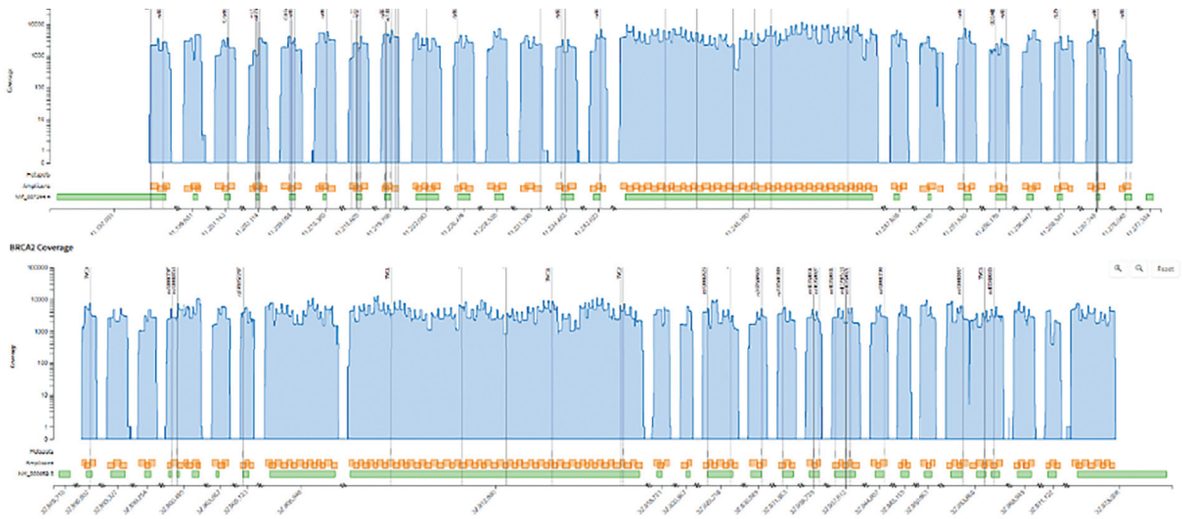


Figure 5: Ion Torrent™ Genexus™ System 3 (2) day workflow in Graz

Monday	Tuesday	Wednesday	Thursday	Friday
sample arrives	sample arrives	sample arrives	sample arrives	sample arrives
sectioning	sectioning	sectioning	sectioning	sectioning
prepare HE	prepare HE	prepare HE	prepare HE	prepare HE
mark tumor area	mark tumor area	mark tumor area	mark tumor area	mark tumor area
macrodissection	macrodissection	macrodissection	macrodissection	macrodissection
digest O/N	digest O/N	digest O/N	digest O/N	digest O/N
extract DNA/RNA	extract DNA/RNA	extract DNA/RNA	extract DNA/RNA	extract DNA/RNA
Genexus O/N	Genexus O/N	Genexus O/N	Genexus O/N	Genexus O/N
techn. Report	techn. Report	techn. Report	techn. Report	techn. Report
final Report	final Report	final Report	final Report	final Report

Speed vs Flexibility Ion Torrent™ Genexus™ System versus Ion GeneStudio™ S5 , presented by Dr. Karl Kashofer, Head of the Laboratory for Diagnostic Genome Analysis, Department of Pathology, Medical University of Graz, Austria.

Dr. Kashofer presented results of comparing performance of their assay on the Genestudio System with the Oncomine Precision Assay on the Genexus System, including an example of a possible week plan of Genexus workflow with 5 batches of samples. In his conclusion he stated that the Ion Torrent Genexus system delivers unprecedented ease of use in a fully automated, ultra-rapid NGS workflow. Cross-validation of the Genexus System against traditional GeneStudio sequencing revealed high concordance of results in conjunction with a substantial decrease of hands-on time.

Reference sample fusion accuracy matrix in Melbourne laboratory

	Reference/Orthogonal Method Results		Total
OPA result	Positive	Negative	
Detected	TP=208	FP=0	208
Not detected	FN=0	TN=169	169
Total	**208**	**169**	**377**

PPV=100%; PPA=100%

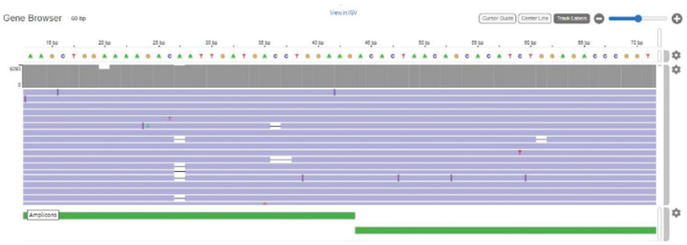


Figure 6. Targeted fusions using Genexus Software Gene Browser - TPM3-NRTK1 in Melbourne laboratory

Peter MacCallum Cancer Centre's validation of a multi-variant NGS assay, Molecular Pathology, Pathology Dept, Peter MacCallum Cancer Centre, Melbourne, Australia, presented by Dr. Chelsee Hewitt.

Dr. Hewitt presented data from her laboratory evaluation of analytical specificity, analytical sensitivity (limit of detection, LOD), accuracy, precision, repeatability, within-laboratory reproducibility, and measurement uncertainty. The Oncomine Precision Assay demonstrated a PPV and PPA of 100% for fusions in replicates of the Seraseq® Fusion Mix sample assessed at 100% and 50% tumor purity. With clinical research samples the PPA was 96% due to OPA not detecting a single fusion, which for several reasons may be difficult to detect using OPA. This fusion would only have been detected by the tiling imbalance method. The breakpoint is close to the 3' end of the *FGFR2* gene (intron 17) and the hypothesised mechanism of activation of this fusion does not require amplification of the transcript.

Clinical research sample fusion accuracy in Melbourne laboratory

	Reference/Orthogonal Method Results		Total
OPA result	Positive	Negative	
Detected	TP=23	FP=0	23
Not detected	FN=1	TN=787	788
Total	**24**	**787**	**811**

PPV=100%; PPA=96%

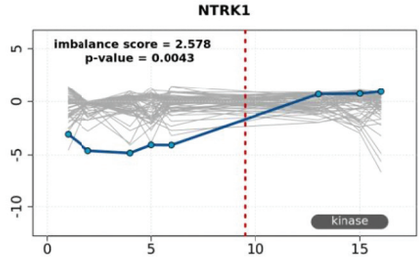


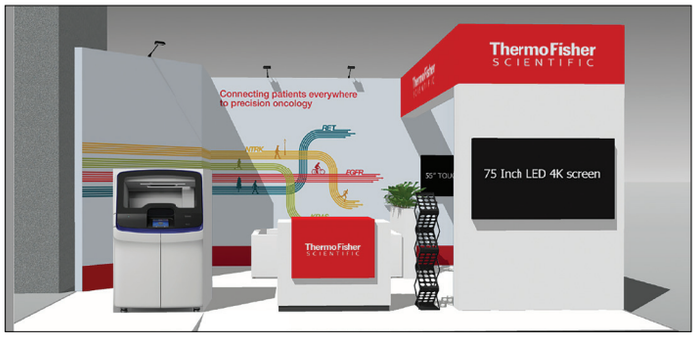
Figure 7. Targeted fusions using Genexus Software Exon Tiling Imbalance Visualisation Tool in Melbourne laboratory

Join us during European Congress of Pathology (ECP) and European Society of Medical Oncology Congress (ESMO) in September 2022.

We will be there with our technical experts and medical affairs teams and look forward to see you at the booth or during one of our educational events:

- ECP Lunch Symposium sponsored by Thermo Fisher Scientific and Bayer on Sunday, 5 September 2022: 13:00–14:30 CEST
- ECP Breakfast Symposium on Monday, 6 September 2022: 7:15–8:15 CEST
- ESMO colloquium sponsored by Thermo Fisher Scientific and Eli-Lilly on Saturday, 10 September 2022: 13:00–14:30 CEST
- ESMO satellite symposium on Saturday, 10 September 2022: 18:30–20:00 CEST at Grenoble Auditorium

You will also be able to catch up with all of our content later, on demand, at www.oncomine.com/ecp2022 and www.oncomine.com/esmo2022.



Read more about the Genexus System at
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