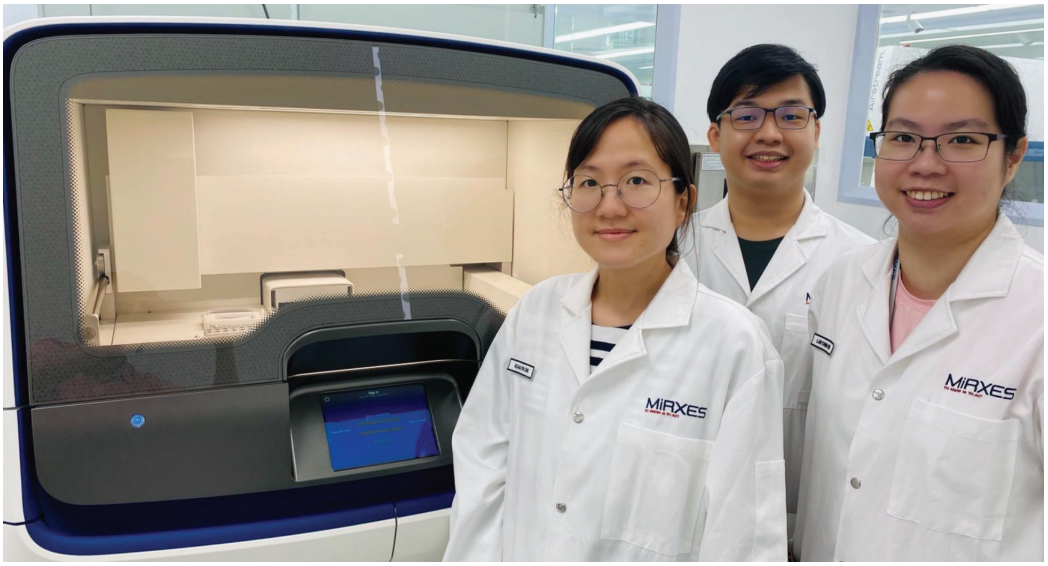
Genexus System news

Issue 6: July 2021

In this issue we are bringing you news on new assays coming on the Genexus System and more new customer experiences from around the world



Experience of M Diagnostic Laboratory in Singapore

Results of a study conducted in Singapore successfully demonstrated that the intended analytical accuracy and performance were consistently achievable. The highly automated workflow will result in significant savings of hands-on time and hence resources. The study demonstrated the ability of the platform to minimize turnaround time significantly help to ensure that results will be available on time. The Ion Torrent™ Genexus™ System is a powerful system that will become a core capability in biomarker detection in oncology clinical research settings.

"The Genexus System has demonstratable prowess in performing automated NGS," said Dr. Maurice Chan, Associate Laboratory Director for M Diagnostics Laboratory, "and will become a leader in oncology clinical research testing, an area where expectations, in terms of testing speed, comprehensiveness, and cost, are rapidly rising."

Announcing the new Ion AmpliSeq SARS-CoV-2 Insight Research Assay

The emergence of several new SARS-CoV-2 variants, including B.1.1.7 and double-mutant B.1.617 that confer increased transmissibility of SARS-CoV-2 virus as well as B.1.351 and P.1 with greater potential for immune escape, has necessitated the urgency to rapidly develop new solutions that can uncover these new variants and associated lineages regardless of sample yield. Thermo Fisher Scientific is pleased to introduce the Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay on the Genexus System to improve SARS-CoV-2 surveillance. The assay is designed to enable early identification of new and known variants from samples that have lower viral loads. By sequencing more than 99 percent of the SARS-CoV-2 genome, the assay covers all potential serotypes and is now available on the Genexus System.

"Quote of the month

"As one of the labs currently sequencing positive SARS-CoV-2 samples as part of the International Travel Testing Programme, we need precise and comprehensive sequencing technology that can help us understand which mutations are present in positive samples for effective monitoring of SARS-CoV-2 cross-border transmission. The new assay offers fast improvement on the sensitivity for lower titer samples, including those from asymptomatic individuals, and has been brilliant for the discovery of emerging variants of concern entering the UK."

– Marco Loddo, Co-Founder, Scientific Director, Oncologica

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In this section we are bringing you testimonials and experiences of new Genexus System and Oncomine Precision Assay users in oncology clinical research, as they presented them at the Oncomine World virtual meeting earlier this year

Dr. Artur Kowalik
Head, Department of Molecular Diagnostics,
Holycross Cancer Center in Kielce, Poland

Dr. Kowalik presented the department's first experience using the Ion Torrent™ Oncomine™ Precision Assay on the Ion Torrent™ Genexus™ Integrated Sequencer for oncology clinical research profiling. They tested non-small cell lung cancer (NSCLC), colorectal cancer, and thyroid cancer samples, comparing the results with results generated previously by the Ion Torrent™ Oncomine™ Focus Assay on the Ion Torrent™ GeneStudio™ sequencer. They were very happy with the data being concordant as expected.

The variants detected in the study included *EGFR* exon 20 deletions, a *MET* exon 14 skipping mutation, *ROS1* fusions, *BRAF* and *EGFR* exon 19 mutations (in one of the colorectal carcinoma samples), *KRAS*, *NRAS,* and *BRAF* wild types (WT), and *Her 2* (*ERBB2*) gene amplification (*ERBB2* CNV ratio = 25.89), which was later confirmed by immunohistochemistry (IHC). In his conclusion, Dr. Kowalik highlighted the automation, ease of operation, and speed of the Genexus Sequencer—they had results in 14 hours.

Leomar Y. Ballester, MD, PhD
Assistant Professor, Molecular Pathology and Neuropathology, and Co-Director, Molecular Diagnostics Laboratory, Department of Pathology and Laboratory Medicine, University of Texas Health Science Center at Houston

In his lab, Dr. Ballester put the Oncomine Precision Assay on the Genexus Sequencer to the test to see how robust, accurate, and fast the results are that they can generate with a particularly small team of three, from FFPE tissue samples.

They used first commercial controls to test detection of SNVs, fusions, and CNVs, detecting successfully 100% of the variants, for 5% MAF (minimal allelic frequency) and higher in the SNV case. (Figure 1).

Gene ID	COSMIC ID	Identifier	HGVS nomenclature	Amino acid	10% AF targeted	5% AF targeted rep 1	5% AF targeted rep 2	2.5% AF targeted
AKT1	COSM33765	Substitution	c.49G>A	p.E17K	18.0%	10.1%	9.0%	4.7%
BRAF	COSM476	Substitution	c.1799T>A	p.V600E	18.7%	7.4%	9.3%	3.4%
EGFR	COSM12378	Insertion	c.2310_2311insGGT	p.D770_N771insG	9.8%	4.4%	2.9%	2.2%
EGFR	COSM6225	Deletion	c.2236_2250del15	p.E746_A750delELREA	11.2%	6.1%	5.5%	2.8%
EGFR	COSM12979	Substitution	c.2573T>G	p.L858R	7.8%	5.7%	5.8%	Not detected
EGFR	COSM6240	Substitution	c.2369C>T	p.T790M	7.4%	3.7%	4.8%	Not detected
FLT3	COSM783	Substitution	c.2503G>T	p.D835Y	16.9%	7.6%	8.8%	3.2%
IDH1	COSM28747	Substitution	c.394C>T	p.R132C	15.9%	6.5%	5.9%	Not detected
KIT	COSM1314	Substitution	c.2447A>T	p.D816V	7.8%	2.9%	8.3%	2.8%
KRAS	COSM521	Substitution	c.35G>A	p.G12D	14.1%	7.7%	8.8%	Not detected
PIK3CA	COSM760	Substitution	c.1624G>A	p.E542K	9.6%	4.9%	7.2%	2.7%
PIK3CA	COSM763	Substitution	c.1633G>A	p.E545K	14.5%	5.9%	7.0%	4.0%
PIK3CA	COSM775	Substitution	c.3140A>G	p.H1047R	13.6%	5.9%	9.5%	2.8%

Figure 1. Reliable detection of SNV at a MAF of 5% or higher, ~75% of SNV detected at a MAF of 2.5%.

Then they tested 28 previously-characterized solid tumor samples, covering 79 distinct genetic alterations across different tumor types and including particularly small samples, achieving 96.2% concordance.

Sample characteristics:

SNV = 49
CNV = 21
Indels = 3
Fusions = 5
RNA exon variant = 1

Solid tumor samples	
Lung adenocarcinoma	10
Colon adenocarcinoma	8
Astrocytomoa/glioblastoma	4
Esophagus adenocarcinoma	1
Lung squamous cell carcinoma	1
Breast adenocarcinoma	2
Melanoma brain metastasis	2
Total	28

In his presentation Dr. Ballester highlighted advantages of using NGS over FISH (Fluorescence In Situ Hybridization) or IHC (Immunohistochemistry), such as its ability to detect mutations as well as amplifications and other types of genomic variants. He pointed out an example of *ERBB2* in colon adenocarcinoma, where clinical research shows over 30% of *ERBB2* variants in colorectal carcinoma (CRC) are short variant alterations, which are not detectable by IHC or FISH, and can be potentially responsive to targeted therapies.

He also demonstrated how the user friendliness of the Genexus Software, which can take you easily from the result view to the Integrative Genomics Viewer (IGV), where you can see the variant should you want to look at the actual reads. See Figure 2.

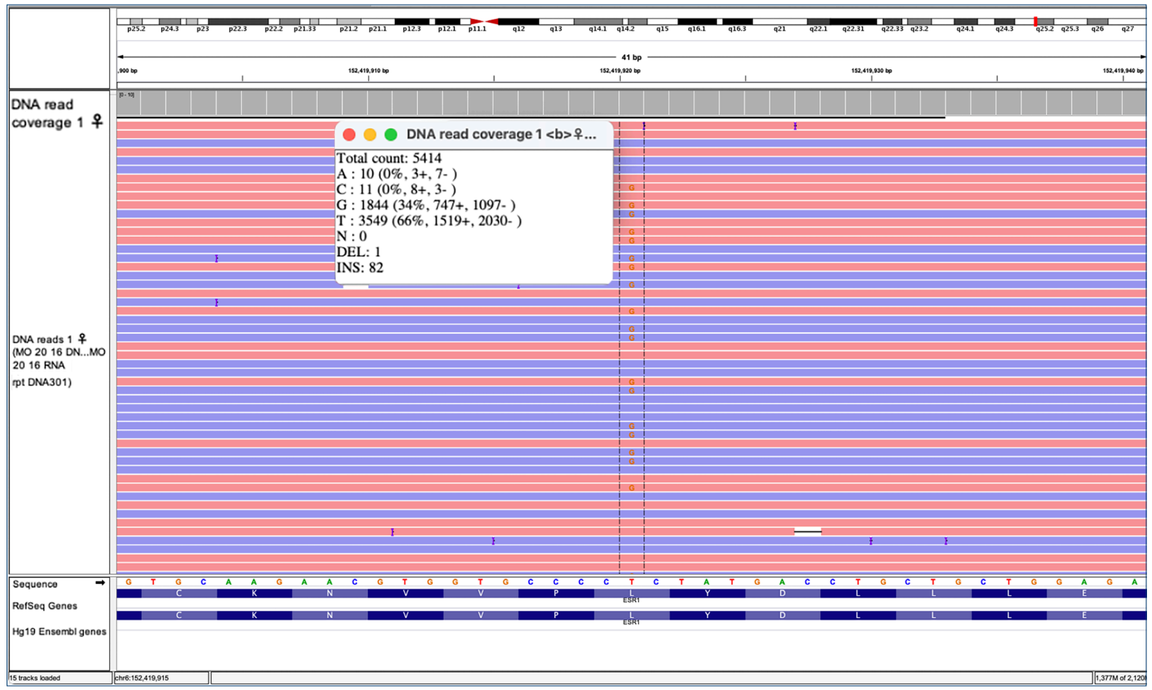

Figure 2. IGV result view with the actual reads.

In his summary, he highlighted that the level of automation, and speed of sequencing and analysis, by the Genexus Sequencer, allowed his laboratory to minimize bench time and reduce turnaround time to as little as 2 days.

At a virtual Manchester Pathology meeting, on July 7, 2021, two new Genexus system and Oncomine Precision Assay users presented results of verification testing and clinical research

Hana Lango Allen, BSc, MSc, PhD
East GLH Scientific Director, NHS Cambridge University Trust in the UK

Dr. Allen has presented results of the Oncomine Precision Assay verification study performed on 3 commercial control materials containing multiple variants and on 62 representative clinical research FFPE tumour samples that were previously characterized, as well as results of 196 clinical research samples tested previously over three months of research.

The success rate has been 99%, DNA input has been low (1–10 ng, crude PK digest, H&E, or IHC stained), overall sensitivity has been high (5% VAF) and coverage has been >2,000x coverage on average.

Overall, she concluded that the verification results and initial clinical research experience showed the Oncomine Precision Assay on the Genexus System to be highly successful in solid cancer sample testing, including challenging tissue samples.

As an example, please see the gene fusion detection results on 20 clinical research samples, which were detected with 100% concordance and included one *ALK* fusion detected by the exon tiling imbalance. (Figure 3).

Fusion driver gene	Fusion expected	Control method	Fusion detected	Concordant	Tumor diagnosis
ALK	ALK rearranged	FISH	EML4(2) - ALK(20)	Yes	Lung adenocarcinoma
	ALK rearranged	FISH	EML4(2) - ALK(20)	Yes	Lung adenocarcinoma
	ALK rearranged	FISH	NPM1(4) - ALK(20)	Yes	Anaplastic large cell lymphoma
	ALK rearranged	FISH	TPM3(7) - ALK(20)	Yes	Inflammatory myofibroblastic tumor
	ALK rearranged	FISH	TPM3(7) - ALK(20)	Yes	Inflammatory myofibroblastic tumor
	ALK rearranged	FISH	ALK expression imbalance	Yes	Lung adenocarcinoma
	ALK-CCDC88A	Sequencing	CCDC88A(12) - ALK(20)	Yes	Malignant hemispheric glioma
BRAF	KIAA1549-BRAF	FISH	KIAA1549(15) - BRAF(9/10)	Yes	Pilocytic astrocytoma
	KIAA1549-BRAF	FISH	KIAA1549(15) - BRAF(9)	Yes	Pilocytic astrocytoma
	KIAA1549-BRAF	FISH	KIAA1549(15) - BRAF(9)	Yes	Pilocytic astrocytoma
	KIAA1549-BRAF	FISH	KIAA1549(15) - BRAF(9)	Yes	Pilocytic astrocytoma
MET	MET ex 14 skipping	Sequencing	MET(13) - MET(15)	Yes	Lung adenocarcinoma
NTRK3	ETV6 rearranged	FISH	ETV6(5) - NTRK3(15)	Yes	Secretory carcinoma of salivary gland
	ETV6 rearranged	FISH	ETV6(5) - NTRK3(15)	Yes	Secretory carcinoma of salivary gland
	ETV6 rearranged	FISH	ETV6(5) - NTRK3(15)	Yes	Secretory carcinoma of salivary gland
	ETV6 rearranged	FISH	ETV6(5) - NTRK3(15)	Yes	Adenocarcinoma
	ETV6 rearranged	FISH	ETV6(5) - NTRK3(15)	Yes	Congenital mesoblastic nephroma
	ETV6-NTRK3	Sequencing	ETV6(5) - NTRK3(15)	Yes	Infantile fibrosarcoma
ROS1	ROS1 rearranged	FISH	CD74(6) - ROS1(34)	Yes	Lung adenocarcinoma
	ROS1 rearranged	FISH & IHC	CD74(6) - ROS1(34)	Yes	Lung adenocarcinoma

Figure 3. 100% concordant for fusion detection in FFPE tumour samples in 20 clinical research cases.

Dr. Réiltín Werner
Chief Medical Scientist in the Molecular Pathology Laboratory at Cork University Hospital in Ireland

Dr. Werner presented results of initial verification for the key lung cancer clinical research variants on 86 anonymized real-world clinical research samples, previously characterized. They also included 45 control samples (commercial controls from Horizon Diagnostics, Seraseq, Acrometrix, and EQA samples).

Both presentations will be available on demand shortly at **oncomine.com/content-center**

AACR 2021 poster: Genexus System specimen-to-report workflow including Genexus Integrated Sequencer and Oncomine Comprehensive Assay v3

Recently, scientists from Thermo Fisher Scientific presented new data at the AACR 2021 annual meeting demonstrating the use of the Genexus System to determine variant calls of FFPE samples using the Ion Torrent™ Oncomine™ Comprehensive Assay v3.

Results show that the variants identified using samples purified from the Genexus System and sequenced on the Genexus Integrated Sequencer were also identified when the same samples were used on the Ion Chef™ Instrument and Ion GeneStudio™ S5 System with 100% sensitivity. A total of 18 variants were detected across 6 DNA and 6 RNA FFPE matched pairs: 17 SNV/INDEL variants and 1 CNV variant. Rare fusion variants were not detected among the 6 samples tested.

The experiment reported illustrates an automated workflow solution for sample purification, library preparation, templating, and sequencing. Variant calling accuracy meets previously established standards for the Ion GeneStudio S5 System (Figure 5). Oncomine Comprehensive Assay v3 was used in this work to demonstrate the speed and ease of use of the system and an equivalent performance to the Ion GeneStudio S5 System (Figures 4 and 5).

RESULTS

Figure 4. Example of an Ion Torrent™ Genexus™ Analysis Report

Purification Samples

Sam... Name	Sample Type	Nucleic Acid Type	Conc.(ng/µl)	QC Conc. Range (ng/µl)	Batch Status	Archive Position	Library Prep
S01_...	FFPE	DNA	12.91	1.11 - 1136.64	Completed	A1	✓
S01_...	FFPE	RNA	105.47	0.95 - 972.8	Completed	B5	✓
S02_...	FFPE	DNA	14.41	1.11 - 1136.64	Completed	A2	✓
S02_...	FFPE	RNA	82.29	0.95 - 972.8	Completed	B6	✓

Variant Calling Report

All | SNVs/Indels | Fusions | CNVs

Oncomine Variants (5.16) Filter Chain Applied
5 of 4,337 Variants

Edit Filters

User Classification	Type	Oncomine Gene Class	Oncomine Variant Class	Gene	Locus	AA Change
Classification	del	Loss-of-Function	Truncating	CDK12	chr17:37627687	p.Pro536HisfsTer74
Classification	ins	Loss-of-Function	Truncating	FANCA	chr16:89831354	p.Trp911SerfsTer11
Classification	snp	Loss-of-Function	Truncating	NOTCH3	chr19:15272024	p.Gln2139Ter
Classification	snp	Loss-of-Function	Truncating	POLE	chr12:133202239	p.Gln2217Ter

Figure 4. Example of an analyis report from Genexus Software.

Sample name	Sample origin	Variant type	Gene	AA change	Genexus System	Ion GeneStudio system	
						Rep 1	Rep 2
FFPE_1	NSCLC tumor	SNV	*BRAF*	p.Gly469Ala	X	X	X
		SNV	*CHEK2*	p.Arg523Cys	X	X	X
		MNV	*NF1*	p.[Leu90=,Glu91Ter]	X	X	X
		SNV	*SETD2*	p.Gln109Ter	X	X	X
		SNV	*SMAD4*	p.Glu390Ter	X	X	X
FFPE_2	NSCLC tumor	SNV	*NF1*	p.Gln543Ter	X	X	X
		SNV	*TP53*	p.Gly245Cys	X	X	X
FFPE_3	NSCLC tumor	SNV	*TERT*	p.[Val197=;Glu198Ter]	X	X	X
		SNV	*CDKN2A*	p.Asp84Tyr	X	X	X
		SNV	*TP53*	p.?	X	X	X
FFPE_4	Colon tumor	SNV	*BRCA1*	p.Lys339ArgfsTer2	X	X	X
		SNV	*FANCA*	p.Trp911SerfsTer11	X	X	X
		SNV	*BRAF*	p.Val600Glu	X	X	X
FFPE_5	Colon tumor	INDEL	*CDKN1B*	p.Ser160PherfsTer44	X	X	X
		SNV	*SMAD4*	p.Arg361His	X	X	X
FFPE_6	Colon tumor	SNV	*BRAF*	p.Val600Glu	X	X	X
		SNV	*CDK12*	p.Arg1356Ter	X	X	X

Sample name	Sample origin	Variant type	Gene	Copy number	Genexus System	Ion GeneStudio system	
						Rep 1	Rep 2
FFPE_3	NSCLC tumor	CNV	*EGFR*	Gain of function	12.36	12.13	12.29
					Total variants	18	18
					False negatives	0	0
					False positives	0	0
					Sensitivity	100%	100%
					PPV	100%	100%

Figure 5. Analysis of 6 unique FFPE samples with the Oncomine Comprehensive Assay v3 using the Ion GeneStudio S5 System. Libraries were created and templated on the Ion Chef Instrument as an orthogonal method.

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

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