Fully automated comprehensive genomic profiling for detection of cancer variants, gene fusions, and complex oncology endpoints

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Introduction

The Ion Torrent™ Genexus™ System has redefined the genomic profiling paradigm as the first fully integrated NGS research platform to provide an automated sample-to-report workflow with next day results. Coupled with the Genexus Purification System, 20 minutes of hands-on time and just two touch points, the Genexus System enables a convenient solution for oncology research. Here we highlight the high-throughput oncology research capabilities of the Genexus System with Oncomine™ Comprehensive Assay Plus GX (OCA Plus), a 500+ gene targeted Ion AmpliSeq™ based research panel.

Methods

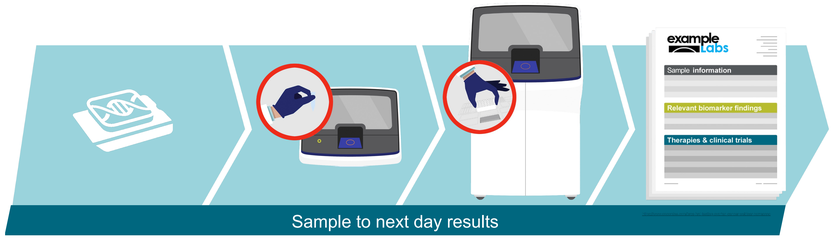
The high-throughput capabilities of the Genexus System enable support for large oncology research panels such as OCA Plus. In addition, Ion AmpliSeq technology enables low sample input as low as 20ng of DNA or RNA. Hence, the extensive per sample coverage and low sample input allows for comprehensive DNA and RNA genomic profiling of relevant cancer biomarkers in over 500 genes including detection of over 1,300 fusion isoforms. We utilized high-molecular weight samples, reference controls, and orthogonally tested FFPE research samples to evaluate DNA variant calling, RNA fusion calling, and key oncology research endpoints.

Single Gene and Complex Biomarkers	
500+ genes	Automated tumor fraction calculation
Small Variants (SNVs and Indels)	Genomic Instability Metric (GIM)
Gene Level Copy Number Variants	Microsatellite Instability (MSI)
Arm-Level Aneuploidy	Tumor Mutational Burden (TMB)
Gene Fusions (>1300 isoforms)	Gene LOH for BRCA1/2 and other HRR genes
MET exon skipping detection at DNA and RNA level	Full coverage of DNA repair pathway genes including HRR and MMR

Oncomine Comprehensive Assay Plus GX
delivers next day CGP results

End-to-end CGP research workflow solution with minimal hands-on time

2 instruments, 1 software workflow solution, and **20 min hands-on** time to generate next day results



Sample	Purification on Genexus Purification Instrument	Library prep, sequencing, analysis on Genexus Sequencing Instrument	Report
As little as 20ng DNA/RNA	10 mins HOT 5 hours runtime	10 mins HOT 26 hours runtime	< 5 mins report generation

The end-to-end NGS workflow is performed by the Genexus System automating the NGS steps with just two touchpoints and 20 minutes of hands-on time (HOT). The Genexus Purification System automates sample preparation by extracting and quantifying nucleic acids within four to five hours. The Genexus Integrated Sequencer automates library preparation, templating, and sequencing with next day results. A single Genexus software ecosystem links the instruments to report. Data files can be exported to use Thermo Fisher Scientific analytic tools like Oncomine Reporter.

SNV/Indel performance in AOHC samples

Variant Type	Sensitivity	PPV
SNVs	99.5%	99.4%
Indels	99.0%	98.5%

The AcroMetrix™ Oncology Hotspot Control (AOHC) was sequenced to evaluate OCA Plus SNV and Indel variant calling performance.

TMB score correlation with TMB Mix controls

TMB Control	Expected (mut/Mb)	Measured (mut/Mb)
TMB-7	7.2 ± 0.2	8.54
TMB-9	9.5 ± 0.4	9.50
TMB-20	20.1 ± 0.2	22.30

Evaluation of TMB score performance by sequencing SeraCare® FFPE TMB Reference Mix samples, with known TMB scores.

MSI performance in Reference Controls and FFPE samples

Reference Controls*		
	Positive	Negative
Positive	48	0
Negative	0	28

	Concordance	Sensitivity	Specificity
TOTAL	100%	100%	100%

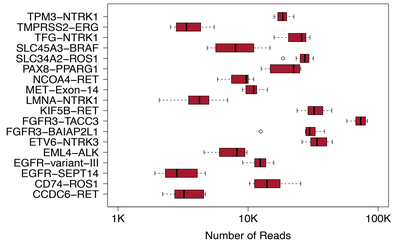
* Reference Controls used HD-830/HD-831/CRL-2577

FFPE Samples		
	Positive	Negative
Positive	23	2
Negative	0	327

	Concordance	Sensitivity	Specificity
TOTAL	99.4%	100%	99.3%

The OCA Plus assay was used to evaluate MSI calls in controls as well as >350 colorectal, endometrial, and stomach FFPE samples. The concordance in FFPE samples was 99.4% with sensitivity of 100% and specificity of 99.3%.

Fusion detection in Reference Controls

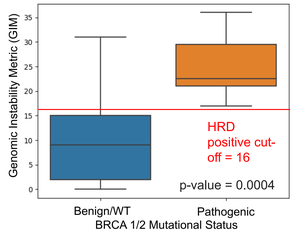


The SeraSeq® Fusion RNA Mix v4 control contains 18 important gene fusions. The OCA Plus assay successfully and reproducibly detects all 18 fusions (2M reads per sample).

GIM analytical performance

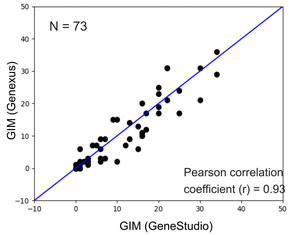
To characterize genomic instability due to Homologous Recombination Deficiency (HRD), we developed a novel quantitative measure called GIM based on unbalanced copy number events. The metric ranges from 0-100, the higher the value, the more genomic instability seen in the sample. For ovarian cancer, we derived a threshold of 16 above which the sample is classified as genomic instability high and vice versa.

GIM can stratify ovarian HRD+ samples



Ovarian cancer samples with BRCA1/2 mutations are HRD positive. GIM can stratify BRCA1/2 mutated samples from BRCA1/2 WT samples.

Correlation of GeneStudio and Genexus Platforms



FFPE samples and cell-lines from different cancer types (N=73) were sequenced on both GeneStudio and Genexus platforms. We found GIM to be highly correlated on the two platforms.

Ovarian Cancer Samples (N=85)	GIM Positive (≥ 16)	GIM Negative (< 16)
Reference GI Positive	47	2
Reference GI Positive	8	28
Positive Precent Agreement (PPA)	95.9	
Negative Precent Agreement (NPA)	77.8	
Overall Agreement (OPA)	88.2	

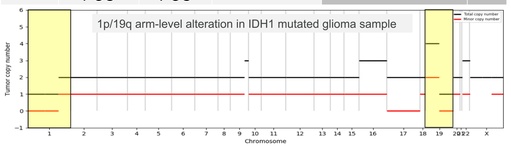
https://www.oncomine.com/brca-hrd-testing-ovarian-cancer-webinar-normanno

Data courtesy of Dr. Normanno, Istituto Nazionale Tumori-IRCCS-Fondazione G. Pascale, Naples, Italy

GIM compared to Genomic Instability (GI) scores from a reference assay shows concordance of 88% in 85 ovarian FFPE samples sequenced on GeneStudio S5.

Examples of gene-level and arm-level CNV calls in breast cancer samples and 1p/19q arm-level alterations in an IDH1 mutated glioma sample.

Sample	ER	PR	HER2	Gene (ERBB2)	ARM
1	80	95	0, FISH NEG	NEG	NEG
2	90	20	3, FISH AMP 10.2	AMP	NEG
3	POS	POS	FISH NEG	NEG	NEG
4	90	90	1-2, FISH AMP	AMP (concomitant gain)	AMP
5	91-100, POS	31-40, POS	0, FISH NEG	NEG	NEG



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