Multifaceted Research Application of Comprehensive Genomic Profiling (CGP) for Detection of Cancer Variants, Gene Fusions, and Complex Oncology Endpoints

Dinesh Cyanam, Geoffrey Lowman, Emily Norris, Michelle Toro, Jennifer Kilzer, Srinivas Nallandhighal, Jianjun Guo, Portia Bernardo, Coleen Nemes, Tanaya Puranik, Yan Zhu, Alex Phan, Derek Wong, Mohit Gupta, Ying Jin, Sameh El-Difrawy, Yu-Ting Tseng, Loni Pickle, Amir Marcovitz, Fatima Zare, Vinay Mittal, Scott Myrand, Santhoshi Bandla, Paul Williams, Eugene Ingerman, Elaine Wong-Ho, Seth Sadis. Thermo Fisher Scientific, Ann Arbor, MI; Carlsbad, CA; South San Francisco, CA; Guilford, CT

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

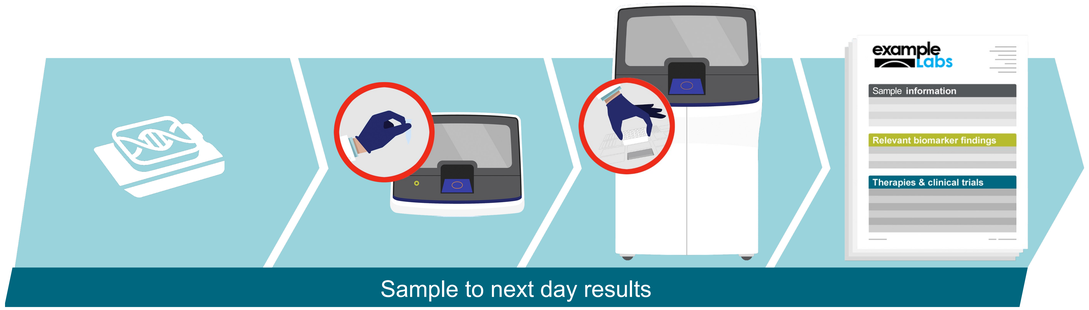
The high-throughput capabilities of the Genexus System enable support for large oncology research panels such as OCA Plus. In addition, Ion AmpliSeq technology enables low sample input of only 20ng of DNA or RNA. Hence, the extensive per sample coverage and low sample input allows for comprehensive DNA 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, FFPE, reference control, and orthogonally tested research samples to evaluate DNA variant calling, RNA fusion calling, and key oncology research endpoints.

Single Gene Biomarkers	Multiple Gene Biomarkers
165 genes with recurrent hotspot mutations	**Cellularity** (Tumor Fraction) calculation
333 genes with focal CNV gains or loss	**Genomic Instability Metric (GIM)** for genomic instability
227 genes with full-coding DNA sequence (CDS)	**MSI-H/MSS** microsatellite markers for Microsatellite Instability (MSI)
46 genes in Homologous Recombination Repair Pathway	**>1 mb** Exonic footprint for **Tumor Mutational Burden (TMB)**
49 fusion driver genes covering >1300 isoforms	**20** genes with loss of heterozygosity **(gene LOH)** for biallelic variant detection in HRR genes
MET exon skipping detection at DNA and RNA level	

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 to generate customizable report formats based on guidelines, clinical trials, curated markers, and novel variants.

RESULTS

SNV/Indel performance in AOHC samples

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

Variant Type	Sensitivity	PPV
SNVs	99.6%	99.2%
Indels	100%	94.4%

TMB score correlation with SeraCare TMB Mix controls

We evaluated TMB score performance by sequencing SeraCare TMB Mix samples, with known TMB scores.

TMB Control	Expected (mut/Mb)	Measured (mut/Mb)
TMB-7	7.2 ± 0.2	9.47
TMB-20	20.1 ± 0.2	21.83

MSI performance in FFPE samples

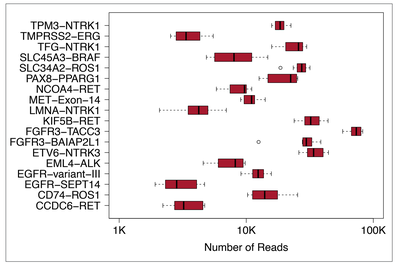
OCA Plus was used to evaluate MSI calls in >350 colorectal, endometrial and stomach FFPE samples. The concordance was 99.4% with a sensitivity of 100% and specificity of 99.3%.

MSI	Orthogonal Samples	
	Positive	**Negative**
Positive	23	2
Negative	0	327

Concordance	Sensitivity	Specificity
99.4%	100%	99.3%

Fusion detection in SeraCare RNA control

The SeraCare RNAv4 control contains 18 important gene fusions. The OCA Plus assay successfully and reproducibly detects all 18 fusions with an average of 2M reads per sample.



ThermoFisher SCIENTIFIC