Accelerating Precision Oncology Research: Automated Comprehensive Genomic Profiling Solution with Next Day Results

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Introduction

Comprehensive genomic profiling (CGP) plays a pivotal role in advancing research in precision medicine. Despite its importance, CGP is often constrained by labor-intensive workflows and extended turnaround times (TAT). To address these challenges, we developed an oncology research panel targeting over 500 genes, using amplicon enrichment and sequencing on the Ion Torrent™ Genexus™ Integrated Sequencer. The Oncomine™ Comprehensive Assay Plus (OCA Plus) enables the detection of single nucleotide variants (SNVs), insertions/deletions (Indels), copy number variations (CNVs), gene fusions, and genomic signatures, including microsatellite instability (MSI), tumor mutation burden (TMB), and homologous recombination deficiency (HRD).

Materials and methods

The OCA Plus assay was optimized for use on the Genexus system, with starting recommendations of 8–30 ng of DNA and RNA input. The system performs automated sample preparation, library preparation, template preparation, sequencing, and variant calling achieving next day TAT. Assay verification employed a combination of 30 cell lines and reference control materials, and over 150 formalin-fixed paraffin embedded (FFPE) research samples of different tumor types to assess sensitivity, specificity, and reproducibility of various end points.

Results

SNV/Indel Performance

SNVs and Indel performance was benchmarked using cancer research FFPE samples, and AcroMetrix™ Oncology Hotspot Control and Seraseq® Tri-Level Tumor Mutation DNA Mix v2 reference controls (*n = 35*). For both SNVs and Indels, the sensitivity was >98% and Specificity was >99% (**Table 1**).

Variant Type	Sensitivity	Specificity
SNVs	98.6%	99.6%
Indels	98.0%	99.0%

Table 1: SNV/Indel Performance

CNV Performance

Evaluation of gene-level CNV gain (CN >=6) and CNV loss (homozygous loss) was performed by sequencing FFPE samples of varying tumor types (*n = 81*) with Affymetrix Oncoscan array as the reference assay. For CNV gain, the sensitivity and specificity were >99.5% and for CNV loss, the sensitivity was 98% and specificity was 99.9% (**Table 2**).

CNV Type	Sensitivity	Specificity
CN Gain	99.6%	99.9%
CN Loss	98.0%	99.9%

Table 2: CNV Performance

Oncomine Comprehensive Assay Plus GX delivers next day CGP results

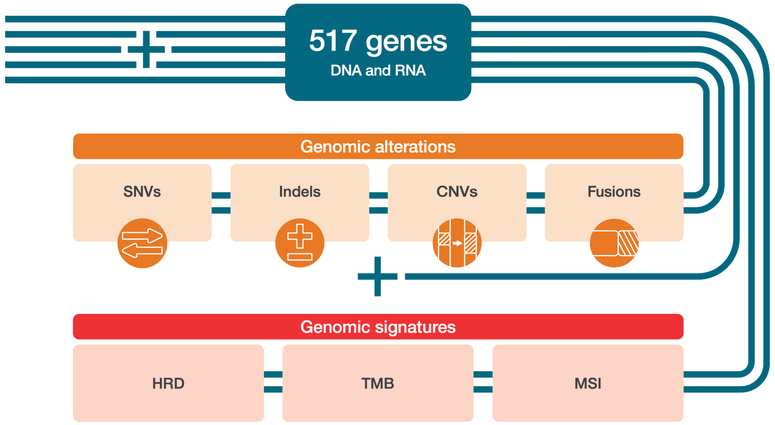

Figure 1: Oncomine Comprehensive Assay Plus GX is a 500+ gene panel measuring genomic alterations such as small variants, CNVs and Fusions (>1300 isoforms). The panel also measures genomic signatures such as HRD, TMB and MSI including automated tumor fraction calculation, BRCA1/2 large genomic rearrangements and mutation signatures.

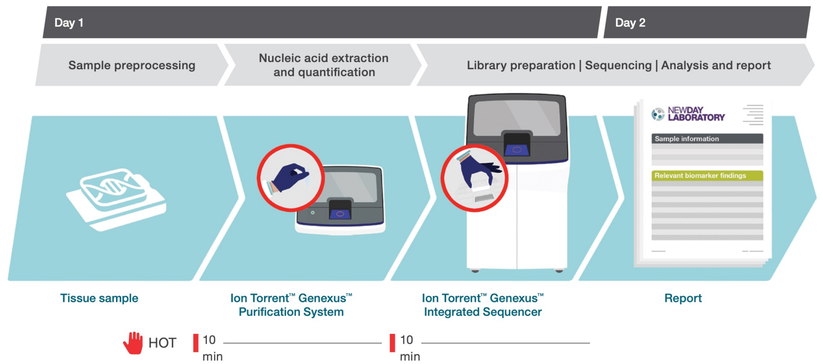

Figure 2: The end-to-end workflow is performed by the Genexus System automating the NGS steps with just two touchpoints and 20 minutes of hands-on time. The Genexus Purification System automates sample prep by extracting and quantifying nucleic acids. The Genexus Integrated Sequencer automates library preparation, templating, and sequencing with next day results.

MSI Performance

MSI performance was tested (*n = 200*) using colorectal, gastric and endometrial research samples and HD-830, CRL-2577 and Seraseq® gDNA MSI-High Mix reference controls. The sensitivity and specificity measured was >99% (**Table 3**).

MSI	Sensitivity	Specificity
Reference Controls	100%	100%
FFPE	100%	99.0%

Table 3: MSI Performance

HRD Performance

Genomic instability metric (GIM) is a novel metric to quantify genomic scars/instability associated with HRD. FFPE samples (*n = 12*) were sequenced on both GeneStudio and Genexus platforms and we found GIM to be highly correlated on the two platforms (**Table 4**).

HRD	PPA	NPA	OPA
FFPE	100%	100%	100%

Table 4: HRD Performance

TMB Performance

TMB concordance was compared to WES, orthogonal assays and Seracare TMB reference controls (*n = 46*) and the Pearson correlation measured was > 0.925 (**Table 5**).

TMB Concordance	Correlation (r^2)
WES	0.954
Orthogonal Assay T	0.925
Reference Controls	0.982

Table 5: TMB Performance

Fusion Performance

Fusion performance was assessed in FFPE samples of varying cancer types (*n = 56*) and in reference controls like Seraseq® NTRK Fusion RNA and Seraseq® Fusion RNA Mix v4 (**Table 6**).

Fusions	Sensitivity	PPV
Reference Controls	100%	100%
FFPE	98.96%	95.28%

Table 6: Fusion Performance

Conclusions

The Oncomine Comprehensive Assay enables accurate characterization of key oncology research endpoints, such as, variant calling, CNV, MSI, GIM and TMB. The Genexus system exemplifies a rapid, flexible, and fully-automated sample-to-report workflow to accelerate research in the field of oncology research.

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