

Increasing the Throughput of NGS Assays for Detection of Oncology Variants, Gene Fusion Events, & Key Oncological Research Endpoints

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INTRODUCTION

The Ion Torrent Genexus System has redefined the genomic profiling paradigm as the first fully-integrated, next-generation sequencing (NGS) research platform to provide a turnkey solution with an automated sample-to-report workflow that delivers results in a single day. The Genexus system launched with the Ion Torrent GX5 chip which delivers ≥48 million reads per chip to enable several oncology research panels. With an increasing need for lower-cost and higher-plexity solutions, we developed a new Genexus chip to increase sequencing throughput. This improved chip delivers over twice the number of reads which better enables key oncology assays for accurate detection of hotspot variants to better investigate important oncological research goals (Figure 1).

METHODS

We demonstrate the high-throughput capabilities of the improved Genexus chip with the OncoPrint Comprehensive Assay Plus (OCA Plus). OCA Plus is a targeted NGS cancer research assay that provides comprehensive genomic DNA and RNA profiling of relevant cancer research biomarkers in over 500 genes including detection of more than 1,300 fusion isoforms across 49 key fusion driver genes. Additionally, OCA Plus has the potential to investigate microsatellite instability (MSI), loss of heterozygosity (LOH), tumor mutational burden (TMB), and homologous recombination repair deficiency (HRD). Performance of OCA Plus on the improved Genexus chip was benchmarked against reference controls and orthogonally validated research samples.



Figure 1. The Improved Genexus Chip for Genexus End-to-End Workflows

RESULTS

The added read capacity of the improved Genexus chip supports research assays such as OCA Plus, which includes >13,000 DNA amplicons to cover >500 genes and 49 RNA fusion drivers. Reference control samples for both DNA and RNA were used to evaluate variant calling, MSI status, TMB score, and fusion calling. All results comprise in-progress development data; endpoint metrics are expected to improve prior to product launch.

Coverage Analysis

OCA Plus shows high coverage uniformity and percentage of amplicons over 350 reads (≥95%) with ≥22M reads per sample (Figure 2, n = 115).

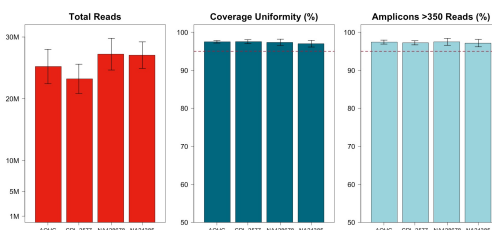


Figure 2. OCA Plus Coverage Analysis Metrics

Variant Calling

AcroMatrix™ Oncology Hotspot Control² (AOHC) samples were evaluated for variant calling performance. The OCA Plus design covers 328 hotspot SNVs across 50 genes within the AOHC control sample. Variant calling results show detection of these variants with mean sensitivity and PPV both ≥95%, while also closely matching the expected allele frequencies (Figure 3, n = 8).

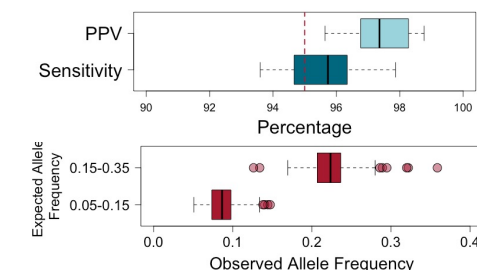


Figure 3. OCA Plus Variant Calling Assessment of AOHC Reference Control

Microsatellite Instability

Samples with known Microsatellite Stable (MSS) or MSI-High status were tested using OCA Plus. OCA Plus accurately calls MSS for >95% of haplotype mapping samples tested (n = 31) and MSI-High for 100% of control samples tested (n = 21) with call accuracy expected to further improve following development (Figure 4).

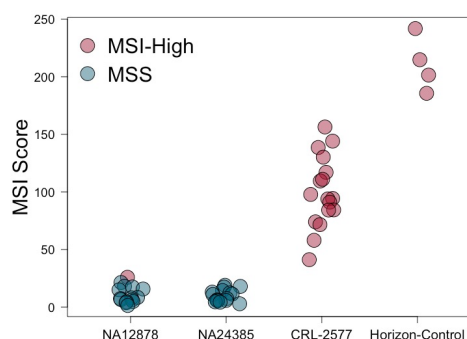


Figure 4. MSI Score and Call for Reference Samples

Tumor Mutational Burden

OCA Plus was used to evaluate TMB score for SeraCare Seraseq® gDNA TMB Mix³ samples that have well-characterized TMB scores. Results utilizing OCA Plus on Genexus show high concordance with the scores evaluated via whole exome sequencing, with a Pearson correlation of 0.95 (Figure 5, n = 4).

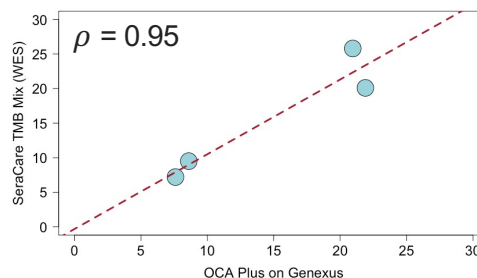


Figure 5. TMB Scores for SeraCare Seraseq® gDNA Reference Control

RNA Fusion Detection

The SeraCare Seraseq® v4 RNA Control⁴ contains 18 gene fusions relevant to cancer research. The OCA Plus assay successfully and reproducibly detects all 18 fusions with an average of 3M reads per sample (Figure 6, n = 8).

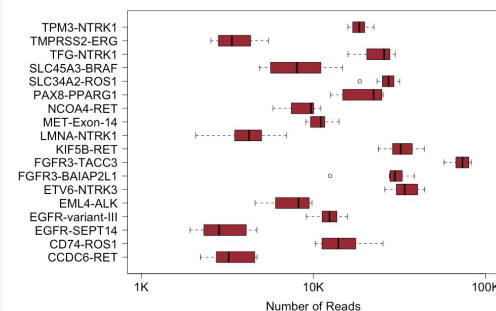


Figure 6. Fusion Detection for SeraCare RNA Control

CONCLUSIONS

The increased throughput of the improved Genexus chip enables comprehensive genomic profiling for research assays such as OCA Plus where an increased number of sequencing reads leads to greater sensitivity for detecting rare variants and low-level fusion transcripts. Further, accurate characterization of key oncology research endpoints, such as MSI, LOH, TMB, and HRD, by NGS solutions can advance studies in immunotherapy research. The Genexus system exemplifies a rapid, flexible, and fully-automated sample-to-report workflow to accelerate research in the field of oncology.

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