High-throughput Next-Generation Sequencing Research Solutions for Detection of Oncology Variants, Gene Fusion Events, and Key Oncology Endpoints

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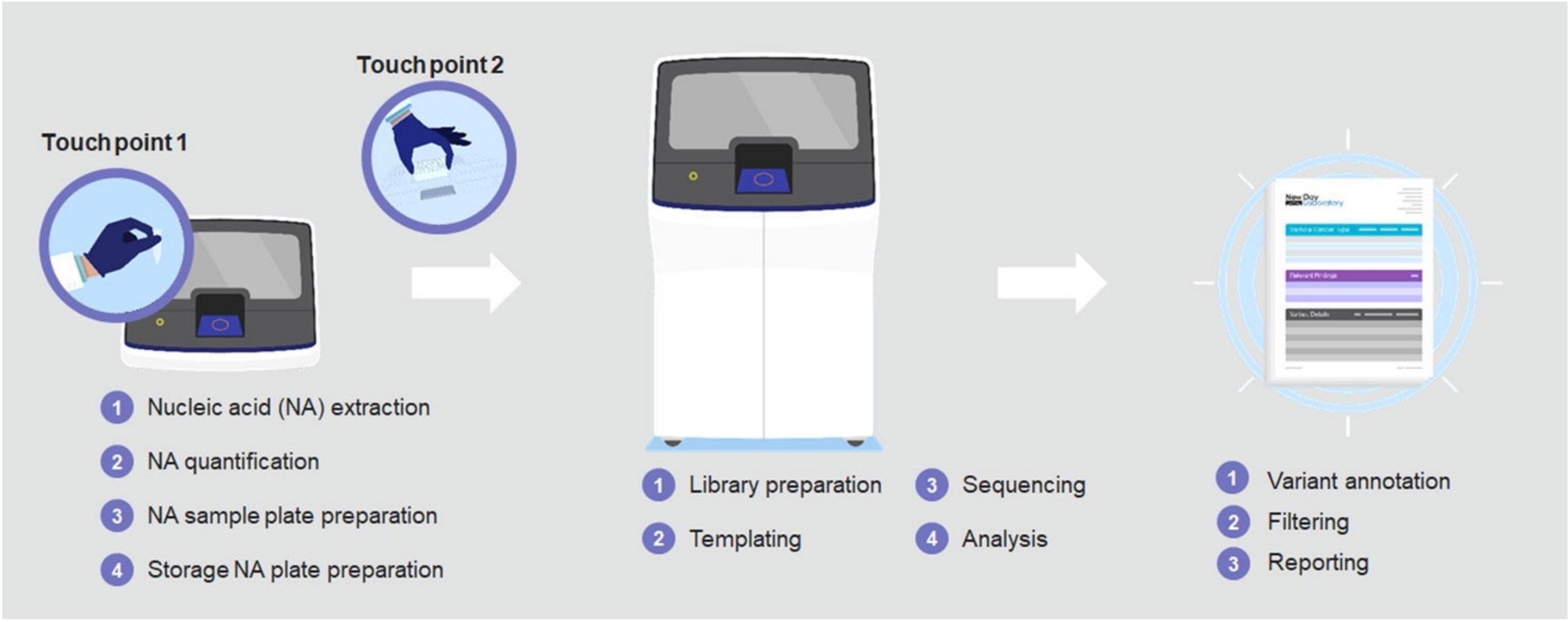
INTRODUCTION

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. With an upstream purification instrument, an enhanced chip architecture to support more reads per sample, and a downstream solution for oncology research reporting, the Genexus System enables a convenient solution for oncology assays. Here we highlight the oncology research applications and high-throughput NGS capabilities of the Genexus System with Oncomine™ Comprehensive Assay Plus (OCA Plus), an oncology research panel that supports DNA and RNA inputs to detect variants, fusions, and evaluate key oncology research endpoints. We demonstrate the ability of OCA Plus on Genexus to evaluate Microsatellite instability (MSI), loss of heterozygosity (LOH), tumor mutational burden (TMB), and homologous recombination repair deficiency (HRD).

METHODS

We demonstrate the high-throughput capabilities of the improved Genexus chip with the Oncomine 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 biomarkers in over 500 genes including detection of >1300 fusion isoforms across 49 key fusion driver genes. The Genexus System uses DNA and/or RNA samples as input, performs purification with the Genexus Purification Instrument, and completes library preparation, sequencing, and bioinformatics analyses with minimal touch points (Figure 1). We utilize high-molecular weight and FFPE samples, reference controls, and orthogonally validated research samples to evaluate performance of OCA Plus Assay on the improved Genexus chip.

Figure 1. Schematic flow-diagram of the Genexus workflow with two instruments connected by one software



The end-to-end NGS workflow is performed by the Genexus System completing and automating NGS steps. The Genexus Purification System replaces sample prep by extracting and quantifying nucleic acids within two or four hours. The Genexus Integrated Sequencer automates library prep, templating, and sequencing. The one ecosystem software links instruments to report. Data files can be exported for third party analytics or use Thermo Fisher analytic tools to generate customizable report formats based on guidelines, clinical trials, curated markers, and novel variants.

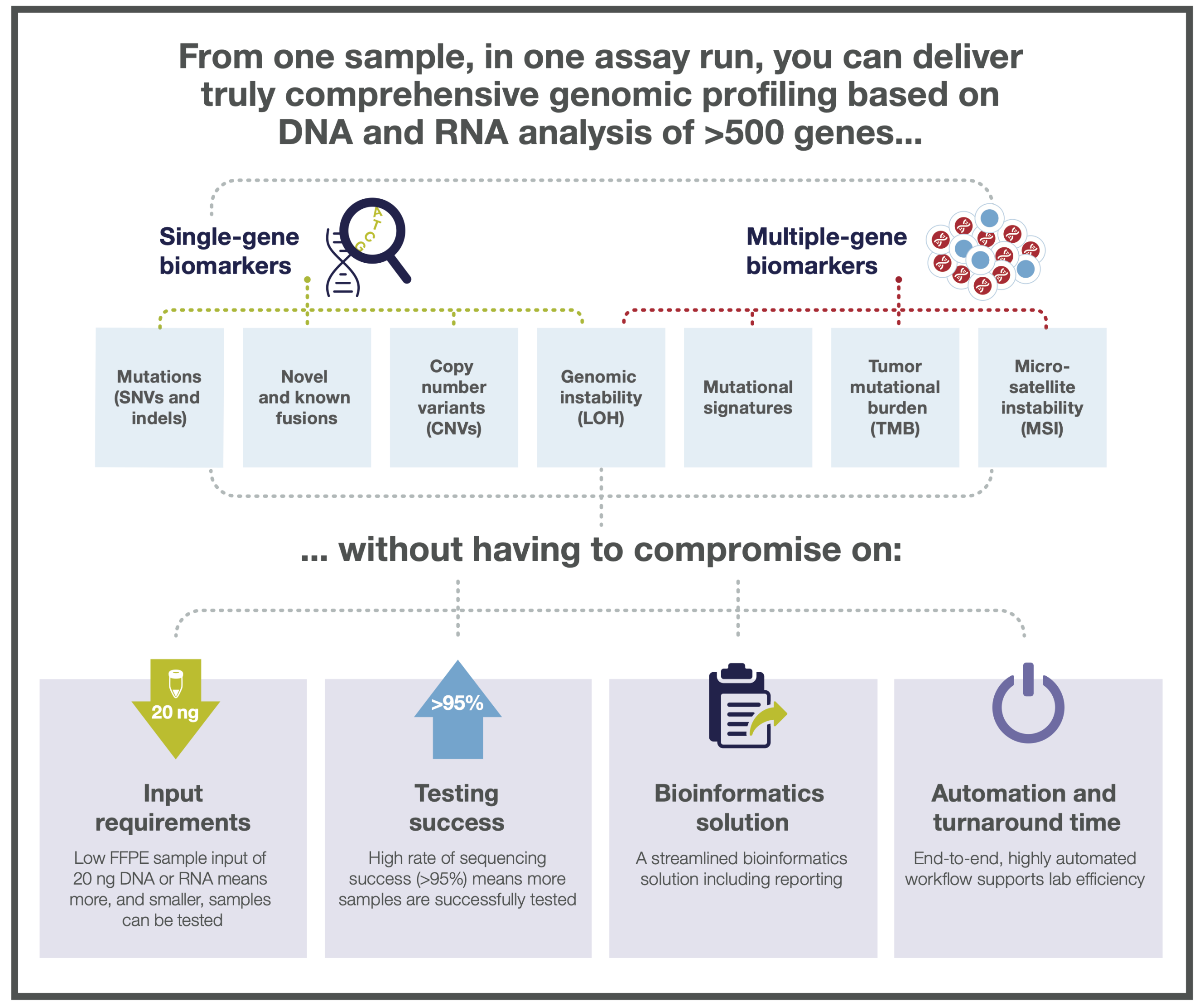
RESULTS

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

Table 1. Summary of the Ion Torrent™ Oncomine™ Comprehensive Assay Plus Content

Single Gene Biomarkers	Multiple Gene Biomarkers
165 genes with recurrent hotspot mutations	**LOH detection**—gene level and sample level
333 genes with focal CNV gains or loss	Analysis and visualization of **mutational signatures**
227 genes with full-coding DNA sequence (CDS)	**>1 mb** Exonic footprint for TMB
49 fusion driver genes covering >1300 isoforms	**MSI-H/MSS** microsatellite markers for MSI detection
MET exon skipping detection at DNA and RNA level	

Figure 2. Oncomine Comprehensive Assay Oncology Endpoints



From one sample, in one assay run, you can deliver truly comprehensive genomic profiling based on DNA and RNA analysis of >500 genes...

Single-gene biomarkers

Multiple-gene biomarkers

... without having to compromise on:

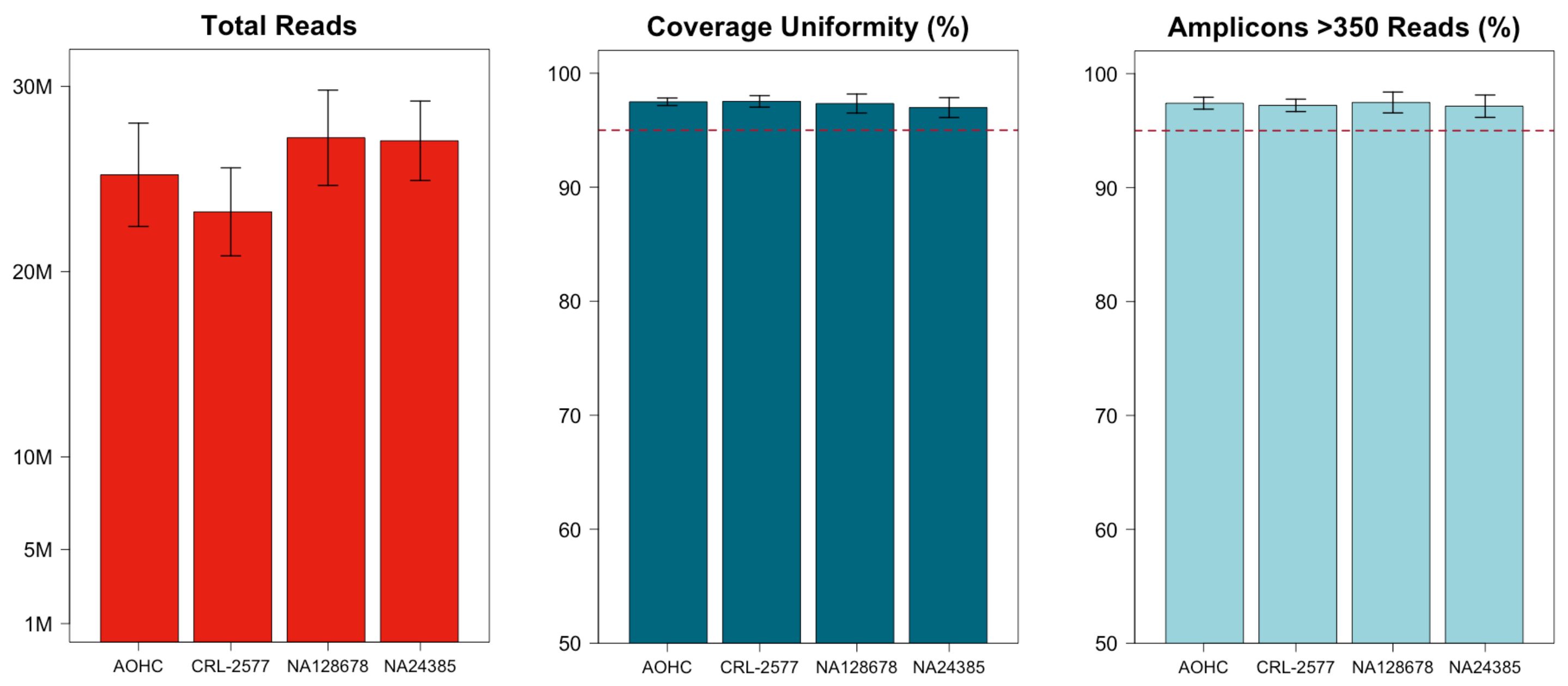
Input requirements
Low FFPE sample input of 20 ng DNA or RNA means more, and smaller, samples can be tested

Testing success
High rate of sequencing success (>95%) means more samples are successfully tested

Bioinformatics solution
A streamlined bioinformatics solution including reporting

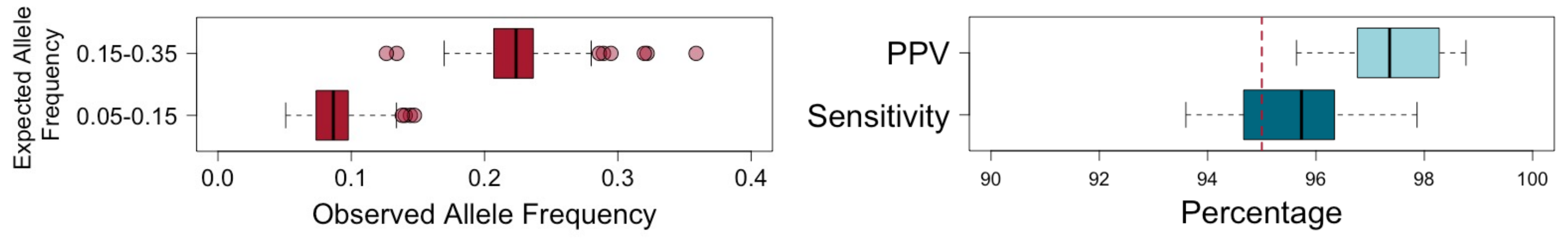
Automation and turnaround time
End-to-end, highly automated workflow supports lab efficiency

Figure 3. OCA Plus Coverage Analysis Metrics



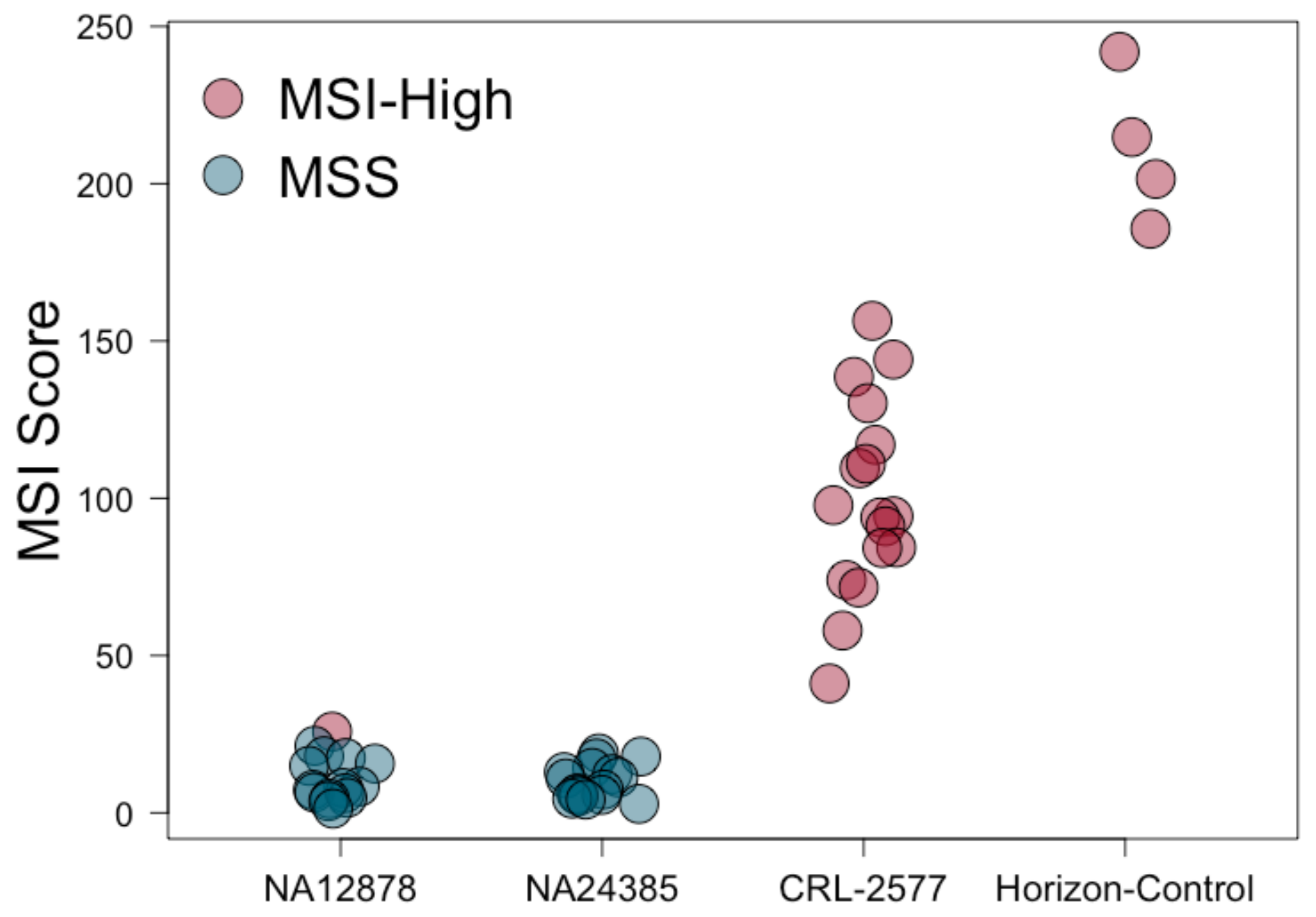
OCA Plus shows high coverage uniformity and percentage of amplicons over 350 reads (≥95%) with ≥22M reads per sample (Figure 3, n = 115).

Figure 4. OCA Plus Variant Calling Assessment on AOHC Reference Control



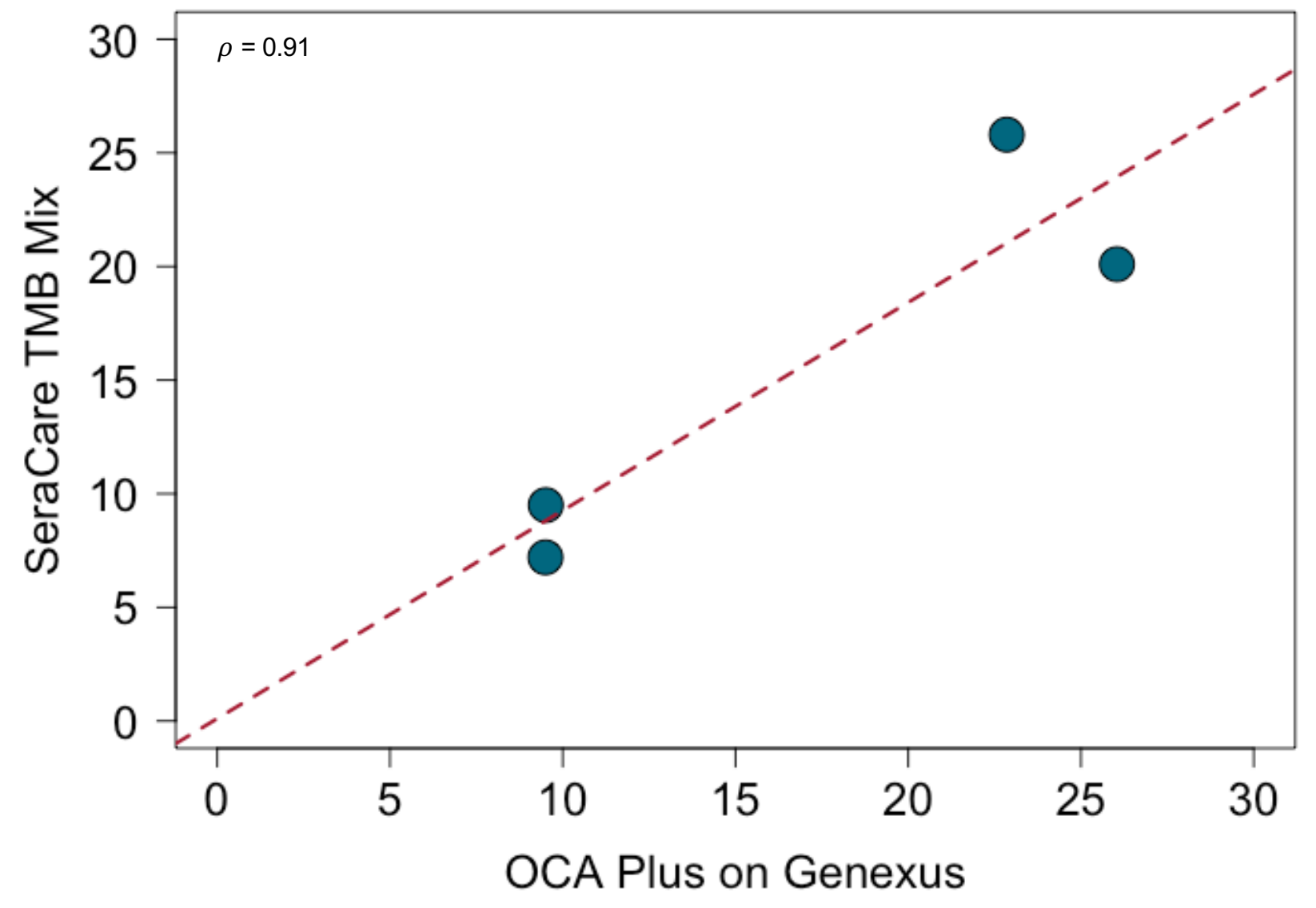
Acrometrix™ Oncology Hotspot Control (AOHC) samples were evaluated for variant calling performance. The OCA Plus design covers 328 hotspot SNVs across 50 genes within the AOHC control sample. Variant calling results show detection of these variants with mean sensitivity and PPV both ≥95%, while also closely matching the expected allele frequencies (Figure 4, n = 8).

Figure 5. MSI Score and Call for Reference Samples



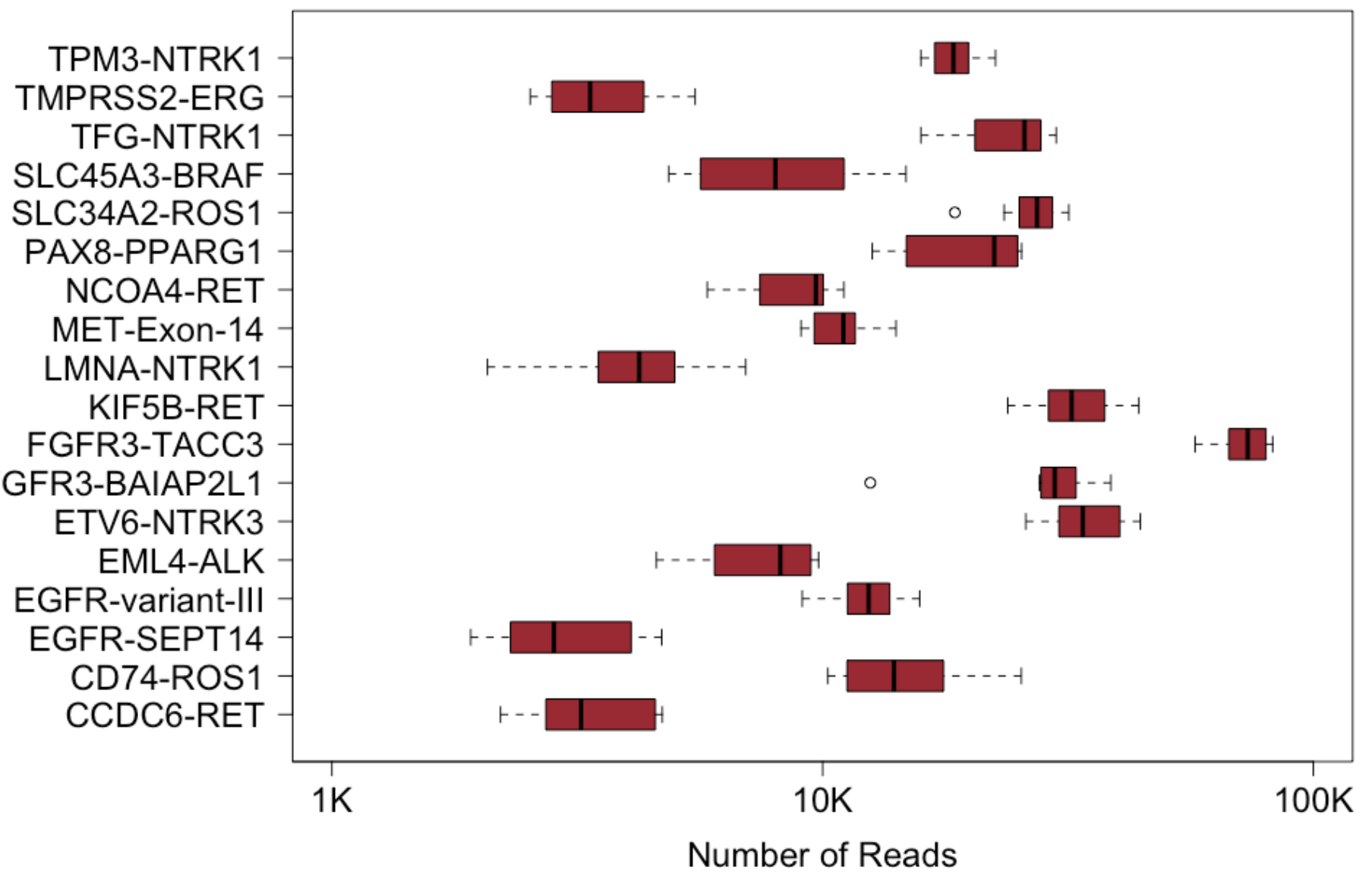
Samples with known Microsatellite Stable (MSS) or MSI-High status were tested using OCA Plus. OCA Plus accurately calls MSS for >95% of haplotype mapping samples tested (n = 31) and MSI-High for 100% of control samples tested (n = 21) with call accuracy expected to further improve following development (Figure 5).

Figure 6. TMB Score Correlation with SeraCare Seraseq gDNA Reference Control



OCA Plus was used to evaluate TMB score for SeraCare Seraseq® gDNA TMB Mix samples that have a known TMB score. Results utilizing OCA Plus on Genexus show high concordance with the expected scores, with a Pearson correlation of 0.91 (Figure 6, n = 4).

Figure 7. Fusion Detection for SeraCare RNA Control



The SeraCare v4 RNA Control contains 18 important gene fusions. The OCA Plus assay successfully and reproducibly detects all 18 fusions with an average of 3M reads per sample (Figure 7, n = 8 samples).

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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