Performance Comparison of the Illumina MiSeq i100 with Established Instruments for Amplicon-Based Somatic and Liquid Biopsy Sequencing Panels

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INTRODUCTION

Introduction. The MiSeq i100 is a newly released benchtop sequencer from Illumina that introduces a range of technical enhancements over the traditional MiSeq platform including significantly reduced runtime, increased throughput, room-temperature storage of consumables, and the use of patterned flow cells for improved cluster density and uniformity. Unlike the non-patterned flow cell design of the legacy MiSeq, the nanowell substrate of the MiSeq i100 enables more consistent cluster generation and higher sequencing efficiency. In this study, we evaluate the performance of the MiSeq i100 against the MiSeq and NextSeq 550 platforms using seven amplicon-based NGS assays targeting somatic and circulating tumor DNA (ctDNA) variants.

Methods. Seven panels were tested: oncoReveal® BRCA1 & BRCA2 Somatic with CNV, oncoReveal® Multi-Cancer v4 with CNV, oncoReveal® Myeloid, oncoReveal® Essential MPN, oncoReveal® Nexus, oncoReveal® Rapid AML, and oncoReveal® Essential LBx. A total of 59 paired samples, including known positives and negatives, were processed using established panel-specific workflows. Sample types included SeraCare and Horizon Discovery reference standards, cancer cell line DNA, and biobanked clinical specimens. Sequencing was conducted over six runs on the MiSeq i100 in 2x121 to 2x251 configurations and benchmarked against results obtained on MiSeq and NextSeq 550 platforms using the same libraries. Variant calling and QC analysis were performed using PiVAT®, with additional metrics compiled via custom scripts. Performance was compared based on mapping rate, on-target rate, coverage uniformity, amplicon-wise mean coverage correlation, and variant detection sensitivity and specificity.

Results. The MiSeq i100 demonstrated a higher mapping rate than MiSeq and NextSeq 550 (99.7% vs 98.5%), but a slightly lower on-target rate (95.5% vs 98.8%), both statistically significant. Panel-level uniformity was slightly lower with MiSeq i100 but not statistically significant. Amplicon coverage patterns were strongly correlated across platforms; however, reduced depth was occasionally observed for longer insert amplicons on the MiSeq i100, with insert length negatively correlated with normalized amplicon depth (Pearson's r = -0.8 to -0.5). GC content showed only a weak correlation to coverage variability (r = 0.14-0.5). All expected true positive variants were detected on the MiSeq i100, and specificity remained comparable across platforms. No false positives were observed. MiSeq i100 usability was enhanced with the streamlined workflow, onboard automation, and room-temperature reagent storage, reducing logistical constraints and improving operational flexibility.

Conclusion. The MiSeq i100 supports the rapid and accurate detection of somatic and liquid biopsy variants with comparable sensitivity and specificity to established Illumina platforms. While slight differences in on-target efficiency and coverage depth for longer amplicons were observed, all panels performed as expected with no impact on variant detection. The instrument's ease of use, shorter runtimes, and reagent handling improvements make it an attractive option for clinical and translational NGS workflows.

MATERIALS AND METHODS

- Seven amplicon-based NGS panels were evaluated (Table 1) across a total of 59 paired samples (known positives and negatives).
- Sample types included reference standards (SeraCare®, Horizon Discovery), cancer cell line DNA, and biobanked clinical specimens.
- Sequencing was performed on the MiSeq i100, MiSeq, and NextSeq 550 platforms.
- MiSeq i100 runs were conducted in 2x121 bp to 2x251 bp configurations across six sequencing runs.
- Each panel was processed using established, panel-specific library preparation workflows.
- Variant calling and QC analysis were conducted using PiVAT®, with supplementary analysis via custom scripts.
- Performance metrics compared included mapping rate, on-target rate, coverage uniformity, amplicon-wise mean coverage correlation, and variant detection sensitivity/specificity.
- Statistical analyses were performed to assess inter-platform differences and correlations with amplicon GC content and insert length.

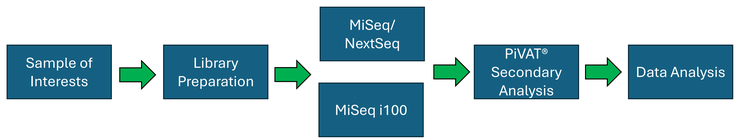


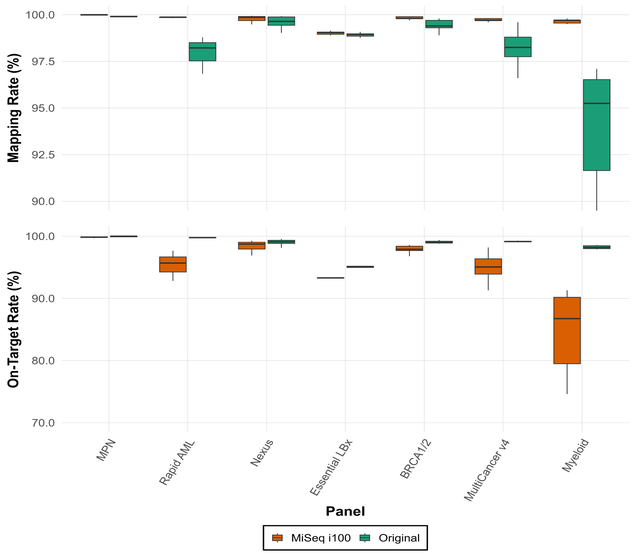
Figure 1. Study design schematic. To minimize noise from other sources, identical library preps were loaded on the three sequencing machines, and the output results were compared across the three.

RESULTS

Table 1. Samples analyzed in this study on seven Pillar amplicon panels. The table describes the samples that were sequenced and analyzed on seven Pillar amplicon panels. Number of amplicons and sum of region of interest for each panel is provided. The number of samples sequenced on each panel is also listed along with the comparator platform.

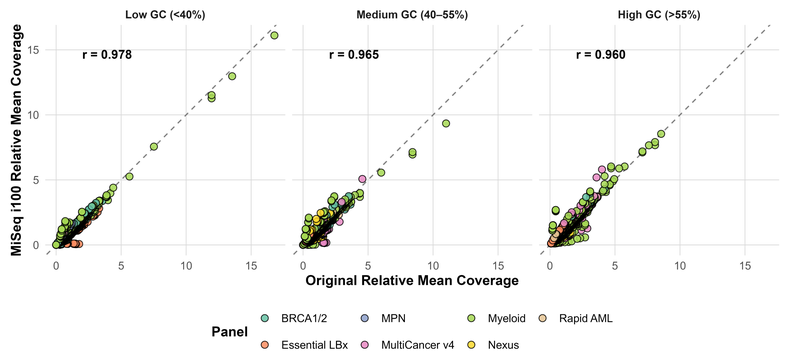
oncoReveal® Panel	# Amplicons	Panel Size	i100 Libraries	Legacy libraries	Comparator platform
BRCA1 & BRCA2 plus CNV	283	19,926	13	13	MiSeq
Multi-Cancer with CNV v4	341	32,807	8	8	MiSeq
Essential MPN	7	1,136	8	8	MiSeq
Myeloid	767	121,685	12	12	MiSeq
Rapid AML	57	9,597	6	6	MiSeq
Nexus 21 Gene	112	12,820	7	7	MiSeq
Essential LBx	201	7,051	5	5	NextSeq 550

Sequencing metric comparisons with i100



(left) Figure 2. Mapping and on-target rate (%) compared between the two platforms. Mapping (top) and on-target rate rate (bottom) percent distributions are shown for each panel. The panels are ordered by their size, from smallest on the left to largest on the right.

(bottom) Figure 3. Comparison of relative per-amplicon mean coverage. Relative per-amplicon mean coverage is shown across amplicons with different %GC. Original relative mean coverage is plotted in x-axis, whereas the i100 coverage is plotted in the y-axis.



RESULTS

Accuracy performance between the platforms

Panel	Sample Type	Paired End Sequencing Read length	i100 True Positive Variant Calls	Legacy Sequencer True Positive Variant Calls
BRCA1 & BRCA2 plus CNV	BRCA Cell Line DNA (BC1)	150	13	13
	BRCA Cell Line DNA (BC13)	150	13	13
	BRCA Cell Line DNA (BC17)	150	17	17
	BRCA Cell Line DNA (BC18)	150	14	14
	BRCA Cell Line DNA (BC20)	150	13	13
Essential MPN	SeraCare Myeloid DNA	150	16	16
Myeloid	Horizon Moderate fcDNA	150	18	18
	SeraCare Myeloid DNA	150	47	47
	Horizon Moderate fcDNA	251	18	18
	SeraCare Myeloid DNA	251	47	47
Nexus 21 Gene	Horizon-Moderate fcDNA	150	34	33
Essential LBx	SeraCare ESR1 cfDNA	121	104	104

Table 2. Accuracy performance between i100 and legacy platforms. Almost identical true positive calls were observed between the two platforms, with an additional call recovery in i100, that was previously missed.

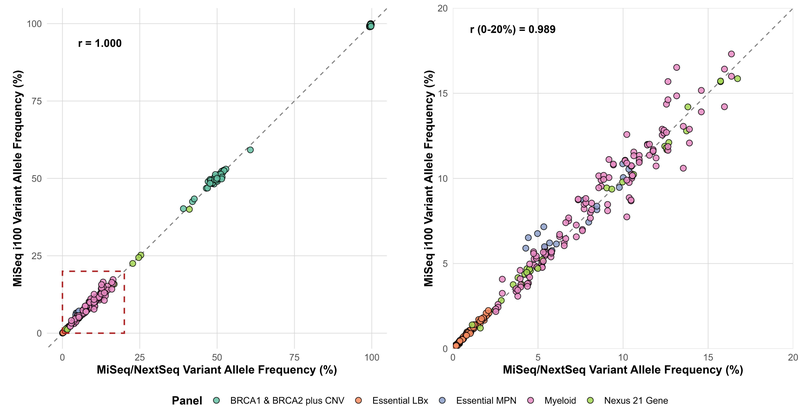


Figure 4. Variant allele frequency (VAF; %) concordance between MiSeq i100 and MiSeq/NextSeq platforms. Scatter plots show the relationship between VAFs measured on the MiSeq i100 (y-axis) and MiSeq/NextSeq instruments (x-axis) across the seven oncoReveal® assay panels. Each point represents a detected true positive variant, with fill color indicating the panel and the dashed diagonal line (y = x) denoting perfect concordance. Left panel: Full-range comparison with a red dashed rectangle highlighting variants with VAF ≤ 20%. Right panel: Expanded view of the 0-20% region illustrating low-frequency variant performance. Pearson's correlation coefficients (r) are shown for both panels, demonstrating strong agreement across the entire VAF range and within the low-frequency subset.

CONCLUSIONS

- MiSeq i100 demonstrates high concordance with the existing Illumina platforms.
- Mapping rates were higher on MiSeq i100 (≈ 99.7%) compared to legacy MiSeq and NextSeq 550 instruments, while on-target rates were slightly lower but not performance-limiting.
- Coverage uniformity and amplicon-level correlation were strong across platforms, confirming consistent sequencing behavior and reliability for clinical and translational workflows.
- Longer amplicons showed modest depth reduction on MiSeq i100, correlating inversely with insert length, whereas GC content had minimal impact on coverage.
- Operational advantages: shorter runtimes, onboard automation, and room-temperature consumables; make the MiSeq i100 a robust, efficient platform for routine NGS testing.