TECHNICAL NOTE

Sequencing performance with NovaSeq™ Control Software v1.8

Highly concordant performance metrics are maintained with NovaSeq Control Software v1.8 compared to v1.7.5

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Introduction

NovaSeq Control Software guides users through sequencing run set up on the NovaSeq 6000 System, controls instrument operations, and displays statistics as a sequencing run progresses. Control software is regularly updated to maintain optimal instrument operation. With the latest update to NovaSeq Control Software v1.8, sequencing performance was evaluated against the previous software version, v1.7.5, across various flow cell and workflow configurations with several different Illumina libraries. This technical note presents the results of that evaluation and demonstrates highly concordant performance, as measured by various primary and secondary metrics.

Methods

Side-by-side sequencing runs were performed with several consumable/workflow configurations and library types.

Library preparation

Multiple libraries were prepared following standard Illumina protocols to evaluate performance across various sample and library types (Table 1).

Sequencing

Prepared libraries were sequenced on the NovaSeq 6000 System using v1.5 Reagent Kits across different flow cells (Table 2).

Table 2: Flow cells used for sequencing[a]

Flow cell	Standard workflow	Xp workflow
SP	Illumina DNA Prep	Illumina DNA Prep TruSeq DNA Nano
S1	TruSeq DNA PCR-Free	N/A
S2	Illumina DNA PCR-Free Prep (16 samples)	Illumina DNA PCR-Free Prep (16 and 24 samples)
S4	TruSeq DNA PCR-Free	Illumina DNA PCR-Free Prep (16 samples)

a. v1.5 reagent kits were used for all flow cells and workflows.

Data analysis

Sequencing results were analyzed and primary metrics, including percent occupancy, percent reads passing filter (PF), yield, intensity, error rate, and Q30, were calculated using Real-Time Analysis Software. Secondary analysis was performed in BaseSpace™ Sequence Hub using the appropriate apps, including the Whole-Genome Sequencing and Variant Calling Assessment Tool apps. Secondary metrics, such as coverage, median fragment length, and recall and precision metrics for single nucleotide polymorphism (SNP) and insertion/deletion (indel) calling were calculated.

JMP 14 analysis software was used for statistical evaluations, including two-sided t-tests and paired t-tests to determine equivalency comparing results using NovaSeq Control Software v1.7.5 and v1.8.

Table 1: Libraries used for evaluation

Library prep kit	Sample	Species	Input	Insert size	Method
TruSeq™ DNA PCR-Free (24 samples)	NA12878	Human	DNA	450 bp	Human WGS
TruSeq DNA Nano (24 samples)	NA12878	Human	DNA	550 bp	Human WGS
Illumina DNA Prep (24 samples)	*B. cereus, E. coli, R. sphaeroides*	Bacteria	DNA	350 bp	*De novo* assembly
Illumina DNA PCR-Free Prep (16 samples)	NA12878	Human	DNA	450 bp	Human WGS
Illumina DNA PCR-Free Prep (24 samples)	NA12878	Human	DNA	450 bp	Human WGS

Results

Primary metrics

Evaluating primary sequencing metrics across flow cells, workflows, and library types showed that most metrics were comparable between NovaSeq Control Software v1.7.5 and v1.8 (Table 3). There were no significant differences in primary metrics for the SP and S1 flow cells. All v1.8 runs with S2 and S4 flow cells showed significant decreases (p-value < 0.05 for two-sided t-test) in intensity across workflows and library types (Table 3). This change is expected due to a recipe change for the S2 and S4 flow cells to increase the minimum sequencing temperature to 25°C. While there was a decrease to intensities observed for S2 and S4, this change is beneficial as it aligns the sequencing temperature with the SP and S1 recipes, increases the sequencing robustness for sequencing in higher humidity, and does not impact other primary or secondary whole-genome sequencing metrics

Secondary metrics

Evaluating secondary sequencing metrics across flow cells, workflows, and library types showed that all metrics were comparable between NovaSeq Control Software v1.7.5 and v1.8 (Table 4). Results from the v1.8 run with the P450-24 plex library sequenced on the S1 flow cell showed marginal decreases in certain metrics (Table 4). These were found to be comparable after reducing the library loading concentration (data not shown).

S2 and S4 sequencing runs with a significant decrease in intensity showed no impact in secondary metrics, including whole-genome sequencing (WGS) coverage and variant calling (Table 4).

Table 3: Summary of primary metric comparisons

	S1, Standard, TruSeq DNA PCR-Free	Summary of v1.8 to v1.7.5 equivalency[a]	S2, Xp workflow, Illumina DNA PCR-Free Prep (16 samples)	S2, Xp workflow, Illumina DNA PCR-Free Prep (24 samples)	S4, Standard, TruSeq DNA PCR-Free Prep	Summary of v1.8 to v1.7.5 equivalency[b]
Metric	Mean difference	Evaluation	Mean difference	Mean difference	Mean difference	Evaluation
% Occupancy	3.45%	Comparable	-0.31%	-0.15%	-0.22%	Comparable
% Reads PF	-1.98%	Comparable	0.09%	0.28%	0.48%	Comparable
Yield R1	-3.73 Gb	Comparable	0.46 Gb	1.18 Gb	2.78 Gb	Comparable
Yield R2	-3.73 Gb	Comparable	0.49 Gb	1.21 Gb	2.79 Gb	Comparable
Intensity R1	-2.39 counts	Comparable	-109 counts	-84 counts	-112 counts	Significant decrease
Intensity R2	-0.29 counts	Comparable	-84 counts	-62 counts	-87 counts	Significant decrease
% Resynthesis	0.20%	Comparable	-0.34%	-0.03%	-0.62%	Comparable
Error rate R1	0.02%	Comparable	0.00%	-0.01%	0.00%	Comparable
Error Rate R2	-0.03%	Comparable	0.00%	0.02%	0.00%	Comparable
Q30 R1	-1.04%	Comparable	-0.02%	0.07%	0.076%	Comparable
Q30 R2	-0.18%	Comparable	-0.17%	0.16%	0.05%	Comparable
Last 10c Q30 R1	-0.84%	Comparable	0.07%	0.47%	0.10%	Comparable
Last 10c Q30 R2	0.16%	Comparable	-0.03%	0.30%	0.14%	Comparable
No. of replicates	10		8	8	10	

a. S1 flow cell.
b. S2/S4 flow cells.

Table 4: Summary of secondary metric comparisons

	S1, Standard TruSeq DNA PCR-Free Prep	S2, Xp workflow Illumina DNA PCR-Free Prep (16 samples)	S2, Xp workflow Illumina DNA PCR-Free (24 samples)	S4, Standard TruSeq DNA PCR-Free Prep	Summary of v1.8 to v1.7.5 equivalency
Metric	Mean difference	Mean difference	Mean difference	Mean difference	Evaluation
Autosome callability	-0.56%	0.007%	0.004%	0.006%	Comparable
Autosome exon callability	-0.041%	0.005%	0.015%	0.009%	Comparable
Coverage at 15×	-0.076%	0.011%	0.011%	0.000%	Comparable
Median fragment length	-7.40 bp	3.00 bp	2.25 bp	2.32 bp	Comparable
Indel pass recall	-0.17%	-0.007%	-0.001%	0.017%	Comparable
Indel pass precision	0.015%	-0.002%	-0.011%	0.013%	Comparable
SNP pass recall	-0.037%	0.005%	0.001%	0.000%	Comparable
SNP pass precision	-0.011%	0.001%	0.000%	0.000%	Comparable
No. of replicates	10	8	8	10	

Summary

This technical note evaluates sequencing performance between NovaSeq Control Software v1.8 and the previous version (v1.7.5). Results demonstrate largely equivalent performance with no impact on variant calling.

Learn more

NovaSeq 6000 System, illumina.com/novaseq

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