Element Biosciences

Technical Note

Enhanced Output with AVITI24™ System

Increase your sequencing output with an AVITI24 Upgrade

Highlights

- AVITI24 System delivers high-quality, affordable sequencing with increased outputs
- An AVITI24 upgrade enables up to a ~50% increase in pass-filter reads for many sequencing applications
- Library loading concentrations can increase slightly (~25%) to achieve up to 1.5 billion reads

Introduction

The AVITI24 System is a first-of-its kind integrated platform offering high-quality, affordable sequencing and multiomic capabilities, all within a single system. Equipped with hardware and compute upgrades, the AVITI24 delivers up to 50% more pass-filter (PF) reads when compared to an AVITI™ System for select kits when running AVITI OS v3.4.0 or later. This increased output is driven by advanced proprietary algorithms incorporated into the system's primary analysis, enabling higher data output at the same cost and with the same run setup workflows.

Library Loading

The AVITI24 System enables a higher sequencing output with the same ease of setup as a standard AVITI sequencing run. To achieve 50% more pass-filter reads on an AVITI24, some libraries need to be loaded at a slightly higher amount as compared to an AVITI run. Element recommends ~1.25x the loading amount used with AVITI as a starting point for an AVITI24 run. Note that if an already optimized library typically yields 1.1-1.2 billion pass-filter reads on an AVITI, there is no need to increase the loading concentration for an AVITI24 run. For details on loading recommendations, see the Cloudbreak Sequencing User Guide.

Run Comparisons

To demonstrate the increased sequencing output delivered on an AVITI24, Element compared primary and secondary metrics between AVITI and AVITI24 Systems across a variety of key applications. Table 1 provides a summary of library types and prep methods used for each assay, along with the loading amount recommended on AVITI24. Use these library loading amounts as a starting point for approximating loading for other similar libraries. Primary quality metrics for each sequencing run are also shown in Table 1.

Assay	WGS		RNA-Seq		scRNA-Seq		Trinity™			
Setting	AVITI	AVITI24	AVITI	AVITI24	AVITI	AVITI24	AVITI	AVITI24	AVITI	AVITI24
Workflow	Elevate™		KAPA Hyper Prep mRNA		10X Chromium Single Cell 3'		Trinity			
Library Type	Elevate PCR-Free		Third Party		Third Party		Elevate - Trinity Panel A		Elevate - Trinity Panel B	
Sample	HG002, HG003, HG004		UHR, HBR		Human PBMC		HG001		HG001	
Recommended Loading	1x	1.25x	1x	1.25x	1x	1.25x	1x	1x	1x	1x
Format	2 x 150		2 x 150		28 + 90		2 x 150 Trinity		2 x 150 Trinity	
PF Reads (M)	863 M	1542 M	1040 M	1530 M	974 M	1500 M	987 M	1562 M	853 M	1247 M
Q30	96.9%	94.6%	95.1%	95.1%	94.3%	96.0%	91.8%	90.5%	94.2%	90.8%

Table 1. Run conditions and primary quality metrics for libraries sequenced on AVITI and AVITI24 System.

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Whole-Genome Sequencing

PCR-free whole-genome sequencing (WGS) libraries were prepared using the Elevate Mechanical Fragmentation Library Prep protocol and kit with 1 µg of HG002, HG003, or HG004 as input and full-length Elevate adapters. A 0.46x/0.62x double-sided SPRI was used for size selection and libraries were fork-collapsed for QC before sequencing. Libraries were sequenced on AVITI and AVITI24 with a 2 x 150 read length. Additionally, libraries were sequenced at higher depth on an AVITI and downsampled to 35x for secondary analysis comparison.

AVITI24 provides a 78% increase in pass-filter reads while still maintaining high quality (Table 1), with 94.6% Q30 (Figure 1A and 1B).

Secondary analysis metrics for WGS samples sequenced with AVITI24 are comparable with AVITI at this higher sequencing output. For the AVITI24 sequencing runs, SNP-F1 scores are ≥ 0.9955 for all samples and INDEL-F1 scores are > 0.9945 for all samples at 35x coverage (Figure 1C and 1D).

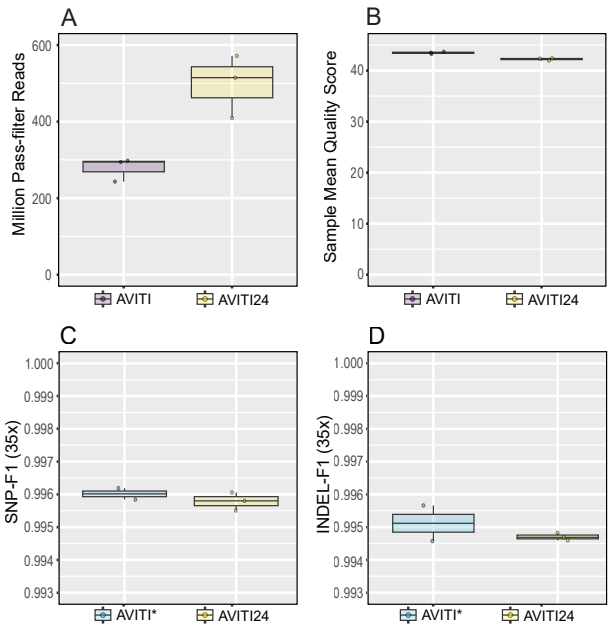

Figure 1. Performance of WGS libraries sequenced on AVITI and AVITI24: (A) Million pass-filter reads per sample, (B) Mean quality score per sample, (C) SNP-F1 score per sample, and (D) INDEL-F1 score per sample. F1-scores at 35x for AVITI libraries in panels C and D were from two higher-depth comparison runs, indicated by an asterisk.

RNA-Seq

RNA-Seq libraries were prepared with the KAPA HyperPrep mRNA kit using 100 ng of either Universal Human Reference (UHR) RNA with ERCC RNA spike-in mix 1 or Human Brain Reference (HBR) RNA with ERCC spike-in mix 2. Five replicates of each sample were pooled after library prep for sequencing.

The library pool was sequenced on AVITI and AVITI24 with a 2 x 75 read length. Data was downsampled to 25 million read pairs per sample for secondary analysis.

AVITI24 provides a 47% increase in pass-filter reads and comparable quality for the RNA-Seq libraries (Table 1). The AVITI24 data also maintains comparable secondary metrics to the AVITI data (Figure 2). Alignment rate varies as expected with the input sample, with > 91.8% uniquely mapped reads from STAR for UHR and > 85.2% for HBR (Figure 2A). Mismatch rate per base also varies with sample but is comparable between sequencing runs (difference of only 0.06-0.07% between AVITI and AVITI24). Gene transcript counts are highly correlated between AVITI and AVITI24 sequencing runs as well, with high Pearson R^2 for UHR and HBR samples (Figure 2B and 2C). At ~1.2 billion reads, AVITI24 enables bulk RNA sequencing of 60 samples at 25 million reads per sample on a single flow cell.

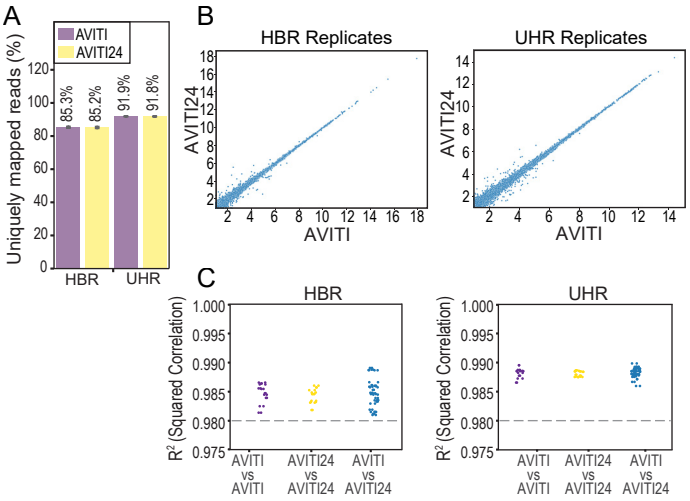

Figure 2. Performance of RNA-Seq libraries sequenced on AVITI and AVITI24: (A) Percent of uniquely mapped reads separated by input (UHR or HBR), (B) correlations per transcript $\log_2$(TPM+1) between AVITI and AVITI24 for UHR and HBR, and (C) R^2 values for AVITI and AVITI24 correlations for UHR and HBR.

scRNA-Seq

Single cell RNA-Seq (scRNA-Seq) libraries were prepared with the 10X Chromium Single Cell 3' Gene Expression library kit using ~10,000 human peripheral blood mononuclear cells (PBMCs). Libraries were end-polished before sequencing on AVITI and AVITI24 with a 28+90 cycle read length. Secondary analysis was performed with Cell Ranger.

AVITI24 provides a 54% increase in pass-filter reads and comparable quality for scRNA-Seq libraries (Table 1). The AVITI24 run also shows comparable secondary metrics to AVITI as calculated by Cell Ranger (Table 2). All Cell Ranger QC metrics thresholds such as valid barcodes and % Q30 bases in various regions were exceeded for both AVITI and AVITI24. Based on the increase in pass-filter reads, AVITI24 enables sequencing of an additional ~4500 cells at the same depth with comparable

mapping rates.

10X Cell Ranger Metric	AVITI	AVITI24
Number of Reads	926,660,702	1,394,237,989
Valid Barcodes	97.5%	97.9%
Estimated Number of Cells	8,374	8,382
Mean Reads Per Cell	110,659	166,337
Reads Mapped Confidently to Genome	94.0%	94.1%
Median Genes per Cell	3,150	3,284
Q30 in Cell Barcode Region	93.5%	96.5%
Q30 in UMI Region	93.5%	95.3%
Q30 in RNA Read	93.7%	95.4%

Table 2. AVITI and AVITI24 performance for ~10,000 human PBMCs analyzed with Cell Ranger.

Trinity

Libraries were prepared for Trinity sequencing with the xGen Exome Sequencing Kit Trinity protocol and the Twist for Element Exome Library Prep and Standard Hybridization with Trinity protocol. Each library prep used 100 ng of HG001 and Elevate full-length adapters. For the IDT xGen Trinity libraries, a pool of 4 µg total DNA was used as input into the hybridization reaction with the IDT xGen Exome panel. For the Twist Trinity libraries, a pool of 2 µg total DNA was used as input into the hybridization reaction with the Twist Exome 2.0+ Comp Spike panel. Both sets of hybridization reactions were sequenced with Trinity on AVITI and AVITI24 with a 2 x 150 read length. Data was downsampled to 25 million read pairs per sample for analysis.

AVITI24 provides a 46–58% increase in pass-filter reads for Trinity libraries while maintaining comparable quality (Table 1) and secondary metrics to Trinity sequencing runs with AVITI. Performance varies as expected by panel, but AVITI24 shows comparable secondary metrics within each tested panel. This includes on-target rate, percent of target bases at 30x coverage, and SNP- and INDEL- F1 scores (Figure 3). At ~1.2 billion reads, AVITI24 enables Trinity sequencing of up to 36 samples at 50x coverage on a single flow cell.

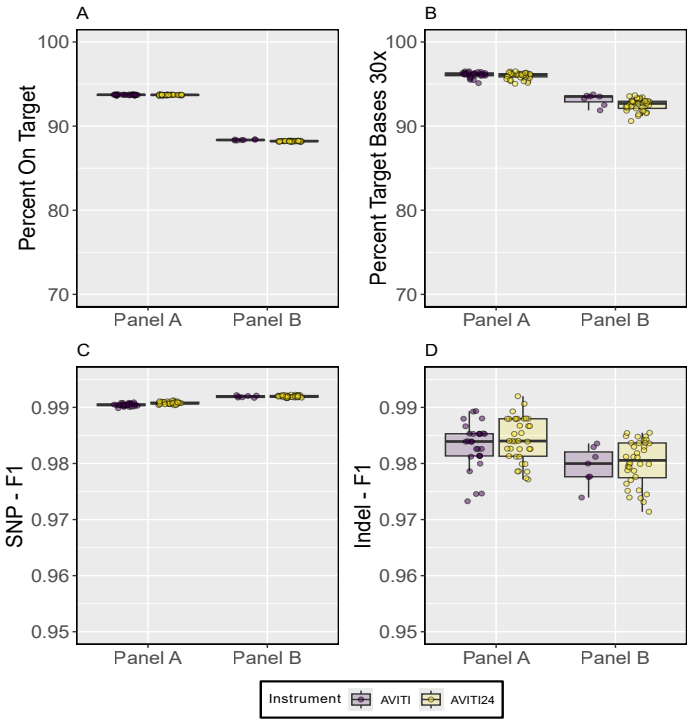

Figure 3. Performance of Trinity libraries sequenced with AVITI and AVITI24: (A) On-target rate, (B) percent of target bases with 30x coverage, (C) SNP-F1 scores and (D) INDEL-F1 scores.

Summary

Along with its multiomic capabilities, AVITI24 delivers high quality, affordable sequencing with increased outputs. Across the applications evaluated, AVITI24 shows an increase in pass-filter reads and comparable performance in secondary analysis at the same kit costs and with no increase in workflow complexity.

Relevant application datasets can be downloaded at AVITI and AVITI24 Enhanced Output Datasets.

To learn more, visit elementbiosciences.com

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