Element Biosciences

Technical Note

Expert Mode HD

Increase your throughput flexibility with AVITI™ Advanced Settings and Expert Mode HD

Introduction

The Element AVITI System, using avidite base chemistry (ABC), provides excellent sequencing throughput and flexibility to fit any assay. The Advanced Run Settings add-on allows experienced users to modify primary analysis and recipe configurations. This add-on includes the Polony Density: High Density setting, known as Expert Mode HD.

Leveraging high-accuracy ABC chemistry, the Expert Mode HD advanced run setting adjusts the polony mapping to enable an increase in run throughput by 20–70% with no change in cost and only a minor quality impact of 1–3% Q30. This setting allows you to boost throughput for certain applications, such as mapping or counting applications (e.g., RNA-Seq, tag or barcode counting) or to consistently meet coverage targets with limited requeues of samples, such as Human Trio or Quad sequencing > 30–35x.

This document describes the quality and output using the Expert Mode HD setting in RNA-Seq, scRNA-Seq, whole-exome sequencing, and whole-genome sequencing with standard reference material.

Run Settings Comparison

Element performed a study to compare primary quality metrics between a sequencing run on the AVITI System in Standard mode versus Expert Mode HD using a variety of assays. Library prep methods used in this study are listed in Table 1.

Expert Mode HD Selection

Expert Mode HD is available in the Advanced Run Settings section of the AVITI run setup screen (Figure 1) under the label Polony Density, which includes two options, Standard (default) and High Density. To apply this setting to your run during run setup, select High Density. Note that after the run has started, this setting is not reversible.

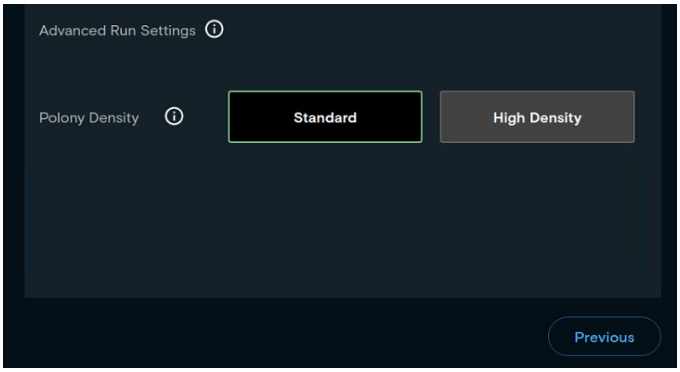

Figure 1. Advanced Run Settings on the AVITI run setup screen.

Standard polony quality filters, using the first 15 cycles of each read, if present, are applied by default or can be modified after the run during FASTQ file generation in Bases2Fastq using the filter-mask flag.

Contact Element Customer Support to enable Advanced Run Settings.

Assay	Sample	Prep Kit	Indexes
RNA-Seq	UHRR	Watchmaker RNA Library Prep Kit	Elevate Long UDI Adapter Kit Set A
scRNA-Seq	Bladder, breast, kidney, ovary tissue, 70,000 cells	10x Chromium Single Cell Expression Flex	10x Chromium Adapter Kit
Human WES	HG001	KAPA HyperPlus Kit	KAPA Unique Dual-Indexed Adapter Set
Human WGS	HG001	KAPA HyperPrep Kit	KAPA Unique Dual-Indexed Adapter Set

Table 1. Library prep methods used in the study

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Run Comparisons

The following table describes the library type, either linear Elevate™ or circular Adept™, and the details for each compared run. All runs were performed with Cloudbreak™ chemistry.

Assay	RNA-Seq		scRNA-Seq		WES		WGS	
Setting	Standard	Expert Mode HD	Standard	Expert Mode HD	Standard	Expert Mode HD	Standard	Expert Mode HD
Library Type	Linear Elevate	Linear Elevate	Circular Adept	Circular Adept	Circular Adept	Circular Adept	Circular Adept	Circular Adept
Loading	6 pM with 0.1 pM PhiX	6 pM with 0.1 pM PhiX	11 pM with 0.1 pM PhiX	11 pM with 0.1 pM PhiX	6 pM with 0.1 pM PhiX	6 pM with 0.1 pM PhiX	10 pM with 0.5 pM PhiX	14 pM with 0.1 pM PhiX
Filter	R1 + R2	R1 + R2	R1 + R2	R1 + R2	R1 + R2	R1 + R2	R1 + R2	R1 + R2
Format	2 x 75	2 x 75	28 + 90	28 + 90	2 x 75	2 x 75	2 x 151	2 x 151
PF Reads	939.8 M	1182.0 M	1178.8 M	1353.1 M	1191.0 M	1332.6 M	1049.0 M	1331.9 M
%Q30	95.4%	93.6%	97.6%	95.8%	95.9%	94.1%	95.1%	92.8%

Table 2. Run conditions and primary quality metrics between Standard mode and Expert Mode HD.

RNA-Seq

An 8-plex of samples of Universal Human Reference RNA (UHRR) including ERCC ExFold Mix 1 or 2 spike-ins (Thermo Fisher) were prepared with the Watchmaker RNA Library Prep Kit with the Elevate Long UDI Adapter Kit Set A, pooled, and sequenced as linear libraries across two flow cells on the AVITI System with either Standard or Expert Mode HD applied. At the same loading concentration, Expert Mode HD yielded 25.9% more pass-filter polonies (1182.0M from 939.8 M, Table 2) with 1.9% lower %Q30 (95.4 to 93.6%).

Secondary analysis metrics from 25 M downsampled reads showed equivalent % unique mapped reads from STAR (Figure 2, A, adjusted p-value 0.171 with Wilcoxon's Test) with a slight increase in mismatch rate, 0.23 vs 0.28% Standard vs. Expert Mode (Figure 2, B, adjusted p-value < 0.001 Wilcoxon's Test).

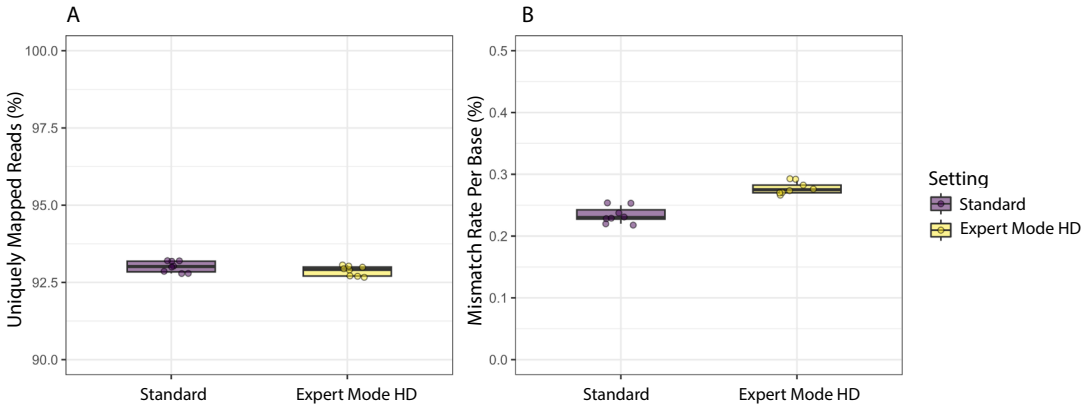

Figure 2. Uniquely mapped reads (A) and Mismatch rate per base (B) from STAR for each setting

Pearson R2 correlation values between runs/settings high and within the variability present in a given run (Figure 3). Correlation between three replicates with each setting (Figure 3, A), within and across setting comparisons, all samples, (Figure 3, B).

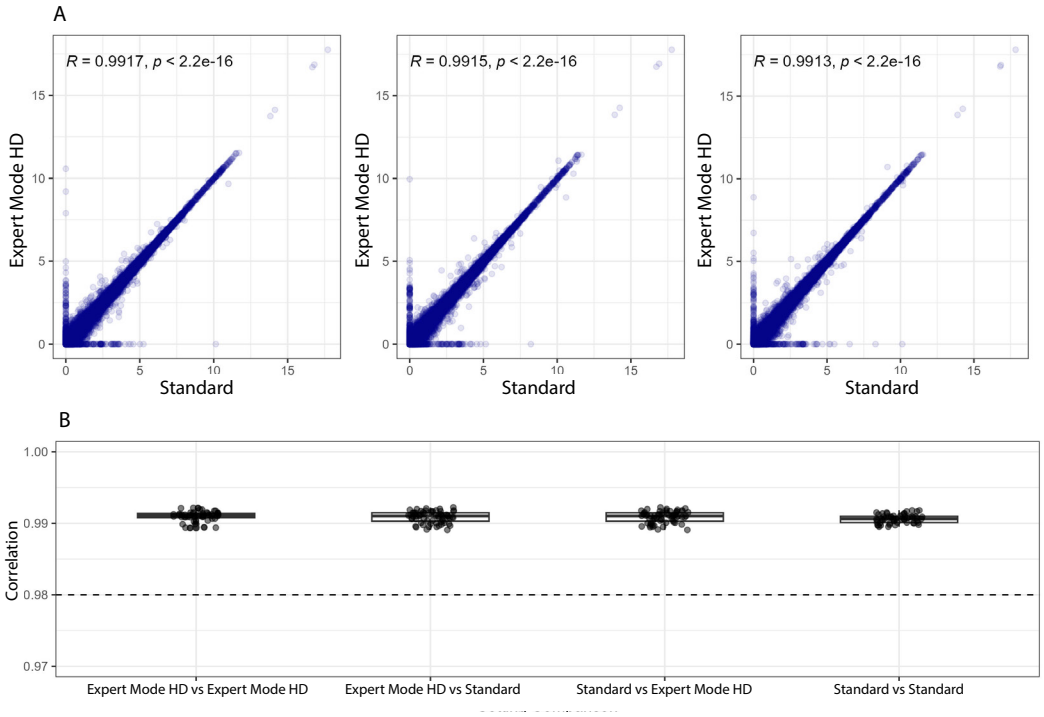

Figure 3. Per setting correlations per transcript $\log_2$(TPM +1).

At 1.2 B total reads, you can sequence an additional 8 bulk RNA-Seq samples at 25 M reads/sample on an AVITI flow cell.

scRNA-Seq

Libraries were prepared by 10x Genomics with the 10x Chromium Single Cell Expression Flex kit using 70,000 total cells across 4 tissues and 16 samples. Libraries were sequenced at Element on one flow cell with 29 + 91 cycles with either the Standard or High Density polony density settings applied to the run (Table 3). Secondary analysis was performed at 10x Genomics with Cell Ranger.

10x Cell Ranger Metric	Standard	Expert Mode HD
Number of reads	1,151,099,092	1,321,127,183
Valid cell barcodes (%)	96.68%	96.61%
Estimated number of cells	69,568	70,288
Reads mapped confidently to filtered probe set	94.96%	94.92%
Median genes detected 5,000 reads/cell	1353	1330
Q30 in the cell barcode region	97.40%	94.60%
Q30 in the multiplexing barcode region	94.30%	92.30%
Q30 in the UMI region	98.30%	96.40%
Q30 in the RNA read	94.40%	92.40%

Table 3. AVITI performance with Standard and Expert Mode HD settings from 70,000 cell samples from 4 tissues analyzed with Cell Ranger

Primary metrics (Table 2) showed 14.8% more PF-polonies with Expert Mode HD (up to 1.35 B) with default quality filter settings with a 1.8% drop in %Q30 (97.6 to 95.8% Q30). All Cell Ranger QC metrics thresholds (i.e., valid barcodes %, Q30 bases in RNA read) were exceeded with both Standard and Expert Mode HD settings. Based on the increase in reads, you can sequence an additional ~10,000 cells at the same depth with comparable mapping rates with Expert Mode HD.

Whole-Exome Sequencing

Libraries were prepared using the Twist Library Preparation Enzymatic Fragmentation (EF) Kit 2.0 with the Twist Exome 2.0 panel from an input of 50 ng of HG001 gDNA. Eight-plex captures (16 total samples) were pooled and sequenced at a 2 x 75 read length on the AVITI with both settings. FASTQ files were generated with Bases2Fastq, downsampled to 40 M reads, aligned with Sentieon BWA, and benchmarked with DeepVariant 1.6 and NIST v4.2.1 truth VCF/BED file.

Primary metrics (Table 2) showed an 11.9% increase in pass filter polonies with Expert Mode HD at the same loading concentration (to 1.33 B polonies) with a 1.9% drop in Q30 (95.9 to 94.1%). Secondary metrics (Figure 4, A-C) showed equivalent on target and Fold-80 base penalties between settings (p-value > 0.05 all comparisons).

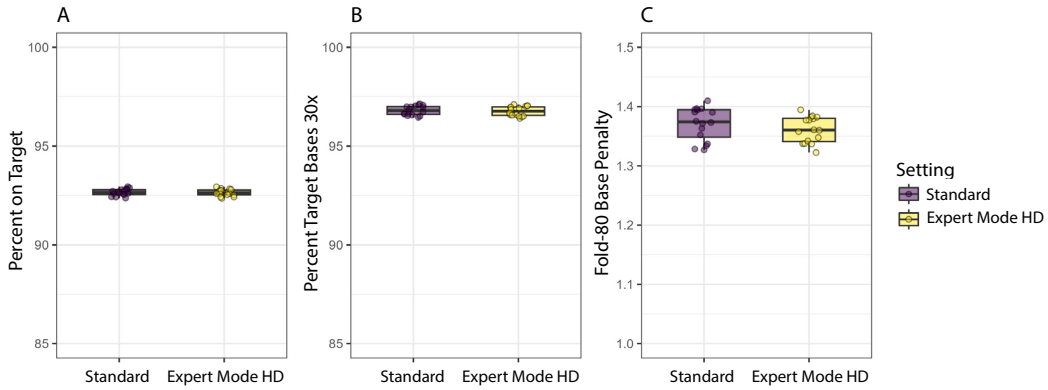

Figure 4. Exome HsMetrics between run settings

For a 36.5 Mb panel with 95% assignment and 90% on-target, you can sequence 35 samples at 100x mean depth with standard throughput. At 1.3 B reads with Expert Mode HD, you can sequence up to 45 samples at 100x coverage with comparable secondary analysis performance. Note that the specifications listed are starting points and there are many considerations when planning your runs.

Whole-Genome Sequencing

PCR-free libraries were prepared from 1 µg of mechanically sheared HG001 reference genomic DNA using the KAPA HyperPlus Kit, end-repaired and ligated with KAPA unique dual index (UDI) adapters, targeting a final library insert size of ~500 bp. Libraries were circularized with the Element Adept Library Compatibility kit v1.1, and sequenced with both settings on an AVITI System at a 2 x 150 read length. Bases2Fastq was used to generate FASTQ files for standard and modified quality filter settings with the --filter-mask option.

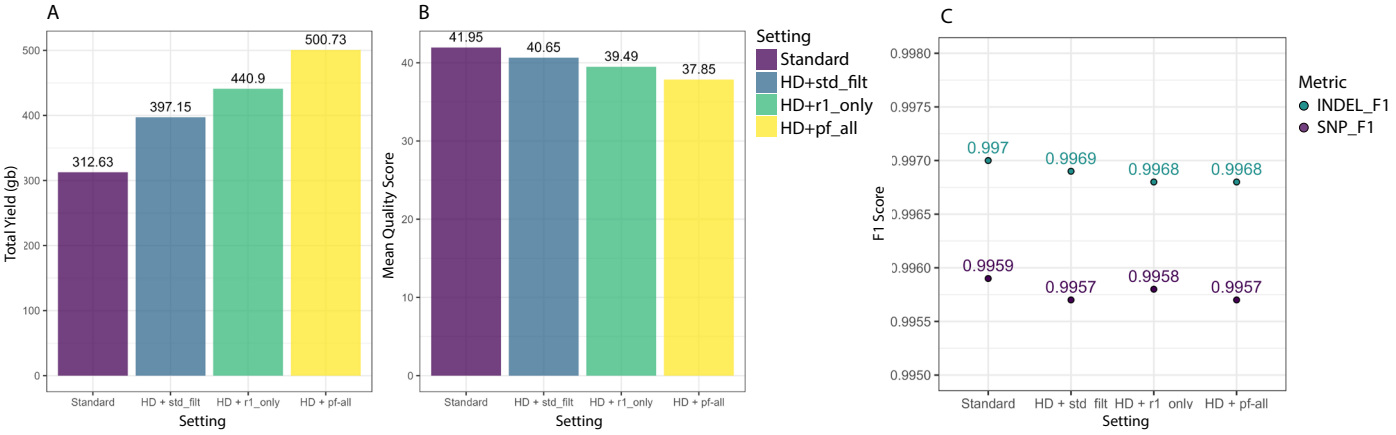

Figure 5. Primary yield and quality score metrics (A and B) between standard run settings and Expert Mode HD with different Bases2Fastq filtering options (std_filt: R1:Y15N*-R2:Y15N*, r1_only: R1:Y15N*-R2:N*, pf-all: R1:N*-R2:N*)

Total flow cell yields with Expert Mode HD with optimal loading were ~400 Gb with standard filtering and up to 500 Gb with 'pf-all' filtering (Figure 5, A). Mean quality scores were > 40 for Expert Mode HD with standard filtering and 37.85 with 'pf-all' filtering (Figure 5, B). Benchmarking for this library at the same downsampling level (35x coverage) for all settings showed a slightly reduced F1 score with Expert Mode HD (0.997 to 0.9968 SNP F1, 0.9959 to 0.9957 Indel F1 with 'pf-all' over standard, Figure 5, C). With 1 B 2 x 150 reads (300 Gb), you can sequence up to 3.27 samples at 30x coverage per flow cell. At 1.5 B reads (450 Gb) possible with Expert Mode HD and altered quality filter settings, you can sequence up to 4.9 samples at 30x coverage or up to 4.21 samples at 35x per flow cell.

A subsequent analysis that passed through additional reads provided with Expert Mode HD (i.e., without downsampling to the same level) showed that these additional reads were of high quality, increasing the SNP-F1 score from 0.997 to 0.9972 and the Indel-F1 score from 0.9959 to 0.997 (Figure 6) and dropping the total number of errors in benchmarking by 15.4% (not shown).

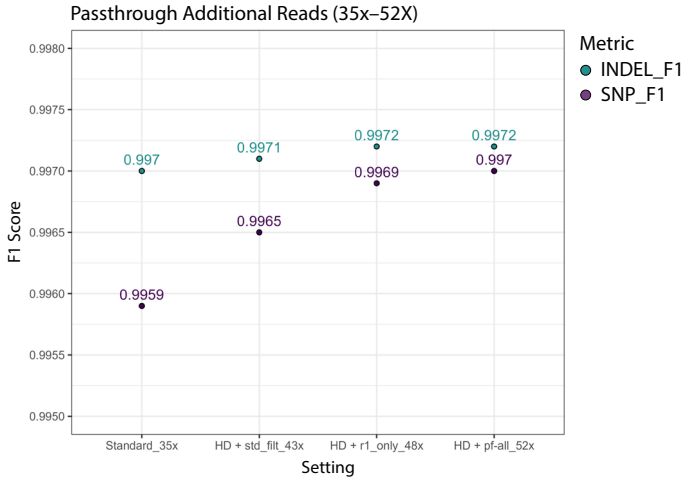

Figure 6. Passing through additional Expert Mode HD reads to benchmarking analysis with HD-Mode. Comparing 35x depth from a standard run to 35x with HD mode (different run), 43x (passing through R1+R2 filtered reads), 48x (R1 only), or 52x (no filtering).

Bases2Fastq filters

For descriptions of available filters, see Bases2Fastq Optional Arguments on the Element website.

Summary

Across the four applications evaluated, results show the use of the High Density polony density setting, or Expert Mode HD advanced run setting, provides consistent increases to density with equivalent or slightly reduced performance in secondary analysis. Owing to the overall high quality of ABC sequencing, the additional reads added using this mode can be leveraged directly into increasing the number of samples or read depth in your WGS, WES, or RNA-Seq applications.

With the flexibility of filtering with Bases2Fastq, you can tune the number and quality of reads that are right for your application. The additional sequencing coverage gained through these settings further drives down costs, solidifying the affordability that the AVITI System provides.

To learn more, visit elementbiosciences.com

Results presented in this technical note are based on sequencing Element libraries. Actual read counts might differ based on lab-specific factors.

Element Biosciences
Telephone: 619.353.0300
Email: info@elembio.com