Authors

Mariana Fitarelli-Kiehl
Senior Applications Scientist

Sarah Trusiak*
Senior Applications Scientist

Sandra Theall
Associate Applications Scientist

Jieqiong Dai
Senior Bioinformatics Scientist

Spencer Debenport
Applications Manager

Alejandro Quiroz-Zárate
Bioinformatics Manager

Rachel Kasinskas
Director – Support & Applications

Sequencing and Life Sciences,
Roche Diagnostics Corporation,
Wilmington, MA, USA

**Author no longer an employee of Roche*

Kelly Blease
Hua Yu
Juan Moreno
Junhua Zhao
Bryan Lajoie
Semyon Kruglyak
Matt Kellinger

Element Biosciences,
San Diego, CA, USA

Date of first publication:
March 7, 2022

Publication date of this version:
March 7, 2022

The KAPA Library Preparation Portfolio allows for high quality sequencing across diverse applications on the Element AVITI™ System

The emergence of novel sequencing technologies has scientists looking to adapt current library preparation workflows to new sequencing platforms. This application note describes the use of KAPA HyperPrep, KAPA HyperPlus, and KAPA EvoPlus Kits to prepare libraries for sequencing on the Element AVITI™ System. Libraries prepared using these kits were made compatible with the Element AVITI™ System using the Element Adept™ Library Compatibility Kit and assessed for multiple applications, including microbial whole-genome sequencing, human whole-genome sequencing, and human whole-exome sequencing. Libraries prepared using KAPA library preparation kits and sequenced on the Element AVITI™ System showed highly uniform coverage across multiple microbial genomes with varying GC content, and enabled variant detection with high sensitivity and precision in both human whole-genome sequencing and human whole-exome sequencing. Thus, these KAPA library preparation kits allow for the generation of high-quality libraries in a diverse set of applications to be sequenced on the Element AVITI™ System.

Introduction

In the past decades, extraordinary progress has been made in sequencing technologies, leading to a significant reduction in per-base cost and increase in throughput and data quality. Access to whole-genome sequencing (WGS) has broadened to many applications in infectious disease, public health and food safety.[1] This progress in sequencing technology has also enabled massive population-level projects for the discovery of new genetic biomarkers in human diseases and traits.[2-3]

The Roche library preparation portfolio offers versatile, highly efficient workflows for a variety of sequencing applications, including WGS, whole-exome sequencing (WES), targeted sequencing, ChIP-Seq, RNA-Seq and Methyl-Seq.[4-9] The KAPA HyperPrep Kit and KAPA HyperPlus Kit include mechanical or enzymatic DNA fragmentation, respectively, providing streamlined and single-tube solutions for the ligation-based construction of sequencing libraries. The recently launched KAPA EvoPlus Kit offers improved enzymatic DNA fragmentation robustness and insensitivity to inhibitors, in a simplified workflow that combines fragmentation and A-tailing in a single step. In addition, all KAPA library preparation kits are validated with the KAPA HyperCap v3.0 target enrichment workflow, which includes several probe panels tailored to different sample types and applications, including human WES.

The rapid improvement in sample preparation methods is driving the emergence of novel sequencing technologies that offer flexibility without sacrificing data quality. In particular, Element Biosciences' benchtop sequencing platform, Element AVITI™ System, uses a novel Avidite™ chemistry enabling a combination of exceptional accuracy, low cost, and operational efficiency. The instrument is designed to operate two independent flow cells and to fit seamlessly into any workflow.

This Application Note describes a new approach for sample preparation and sequencing by combining KAPA library preparation workflows from Roche with the sequencing platform and chemistry from Element Biosciences. The performance of this combined workflow was evaluated for bacterial WGS, human WES, and human WGS.

Materials and Methods

This study was designed to demonstrate the compatibility of KAPA library preparation kits with the Element AVITI™ System (Element Biosciences). The performance of this workflow was tested across three applications: microbial WGS, human WGS, and human WES. A summary of the experimental design is given in **Figure 1**.

1. Microbial WGS

Input Sample: In this study, three bacterial species relevant for human health were selected to represent a wide range of genomic GC contents: *Clostridium difficile* (29% GC), *Escherichia coli* (51% GC), and *Bordetella pertussis* (68% GC). Bacterial genomic DNA was obtained from the American Type Culture Collection (ATCC). Strains and accession numbers were as follows: *C. difficile* (Hall and O'Toole) Prevot, strain 630 (BAA-1382D-5); *E. coli* (Migula) Castellani and Chalmers, strain MG1655 (700926D-5) and *B. pertussis* (Bergey, et al.) Moreno-Lopez, strain Tohama 1 (BAA-589D-5). The genomic DNA of each bacterial species was equally mixed by mass, and 100 ng of the resulting mixed DNA was used as input into library preparation.

Library Preparation with KAPA EvoPlus Kit: Triplicate libraries were prepared with the KAPA EvoPlus Kit with enzymatic fragmentation for 15 min at 37°C to target a mode fragment length of 300 bp. Indexing was incorporated during the adapter ligation step with KAPA Unique Dual-Index Adapters. Adapters were used as recommended in the Instructions for Use (IFU) (15 µM for 100 ng input). Libraries were amplified with 5 cycles of PCR to achieve a minimum of 0.5 pmol (16.67 nM) concentration for downstream sequencing.

Library Preparation with KAPA HyperPlus Kit: Triplicate libraries were prepared with the KAPA HyperPlus Kit with enzymatic fragmentation for 25 min at 37°C to target a mode fragment length of 300 bp. Indexing was incorporated during the adapter ligation step with KAPA Unique Dual-Index Adapters. Adapters were used at the IFU-recommended concentration of 15 µM for 100 ng inputs. Libraries were amplified with 5 cycles of PCR to achieve a minimum 0.5 pmol (16.67 nM) concentration for downstream sequencing.

	Starting material	Library preparation	Library circularization	Sequencing	Data analysis
Microbial WGS	100 ng of bacterial gDNA (*Clostridium difficile, Escherichia coli,* and *Bordetella pertussis* mixed)	KAPA EvoPlus Kit ▪ KAPA UDI Adapters (5 PCR cycles) KAPA HyperPlus Kit ▪ KAPA UDI Adapters (5 PCR cycles)	Element Adept™ Library Compatibility Workflow	Element AVITI™ System	▪ Data subsampling: 6M reads ▪ Metrics: GC-bias
Human WES	100 ng of human gDNA (NA12878)	KAPA EvoPlus Kit ▪ KAPA Universal Adapters and UDI Primers (6 PCR cycles) ▪ TE with KAPA HyperExome (8 PCR cycles) KAPA HyperPlus Kit ▪ KAPA Universal Adapters and UDI Primers (6 PCR cycles) ▪ TE with KAPA HyperExome (8 PCR cycles)	Element Adept™ Library Compatibility Workflow	Element AVITI™ System	▪ Data subsampling: 60M reads ▪ Metrics: Mean Coverage, Percent on-target reads, Fold 80 base penalty, Percent duplicate reads, depth of coverage, GC-bias, and SNV and INDEL calling
Human WGS	1 µg of human gDNA (NA24385)	KAPA HyperPrep Kit ▪ KAPA UDI Adapters (PCR-free)	Element Adept™ Library Compatibility Workflow	Element AVITI™ System	▪ Data subsampling: 360M reads ▪ Metrics: GC-bias, and SNV and INDEL calling
	100 ng of human gDNA (NA12878)	KAPA HyperPlus Kit ▪ KAPA UDI Adapters (5 PCR cycles)			

Figure 1. Experimental outline for microbial WGS, human WES, and human WGS.

Final Library QC: All libraries were quantified after the post-amplification cleanup with the qPCR-based KAPA Library Quantification Kit for Illumina platforms (Roche). Library size distributions were confirmed with a 2100 Bioanalyzer instrument and Agilent High Sensitivity DNA Kit (Agilent Technologies).

Library Preparation with the Element Adept™ Library Compatibility Workflow: The final KAPA EvoPlus and KAPA HyperPlus libraries prepared in the above steps were made compatible with the Element AVITI™ System (Element Biosciences) as individual libraries. An amount of 0.5 pmol (30 µl of 16.67 nM) of each linear library was circularized using the Element Adept™ Library Compatibility Kit (Element Biosciences). After circularization and cleanup, library concentrations were analyzed by qPCR with the standard and primer mix provided in the Adept™ Kit. The resultant libraries were denatured and sequenced on the Element AVITI™ System.

Data Analysis: Adapters were trimmed during demultiplexing using bcl2fast (v2.20.0.422). The quality trimming was performed using fastp (v0.19.3). All reads were primarily aligned to the aggregated reference genome of the three organisms of interest: *Escherichia coli* (ATCC 700926), *Clostridioides difficile* (ATCC BAA-1382), and *Bordetella pertussis* (ATCC BAA-589) using BWA MEM v0.7.17. Afterward, alignments were split by each organism and proceeded with the following analysis.

Alignment was evaluated using samtools v.1.9. GC-bias was analyzed using Picard CollectGcBiasMetrics v2.26.3. Start-site complexity was analyzed over a 50 bp window (-10 to +40 bp relative to the alignment start site). Coverage depth was analyzed using Mosdepth v0.2.6, with libraries down-sampled to 6M total raw reads (3M read pairs). Microbial genomes were assembled according to the method described by Bronwen Miller et al[4] using Spades v3.13. The assembly was evaluated using Quast v5.0.2.

2. Human WES

Input Sample: Unique dual-indexed libraries were prepared from 100 ng of high-quality human genomic DNA (HapMap sample NA12878, Coriell Institute for Medical Research) with the KAPA EvoPlus and KAPA HyperPlus Kits.

Library Preparation with KAPA EvoPlus Kit: Triplicate libraries were constructed with the KAPA EvoPlus Kit, with enzymatic DNA fragmentation for 20 min at 37ºC. Libraries were barcoded by ligation to KAPA Universal Adapters (adapters at final concentration of 1.36 µM) and subsequent 6 cycles of pre-capture amplification using KAPA Unique Dual-Indexed (UDI) Primer Mixes.

Library Preparation with KAPA HyperPlus Kit: Another set of triplicate libraries was prepared with the KAPA HyperPlus Kit, using enzymatic DNA fragmentation for 25 min at 37ºC. Ligation was performed with the KAPA Universal Adapters at final concentration of 1 µM, and sample indexing was completed with 6 cycles of pre-capture amplification using KAPA UDI Primer Mixes.

Pre-capture Library QC: The concentration and size distribution of the resulting pre-capture libraries were determined with the Qubit dsDNA HS Assay kit (Invitrogen) and a 2100 Bioanalyzer instrument and Agilent High Sensitivity DNA Kit (Agilent Technologies).

Target Enrichment: Both KAPA EvoPlus and KAPA HyperPlus libraries were target-enriched with KAPA HyperExome probes in single-plex reactions, following the KAPA HyperCap Workflow v3.0 instructions for a capture target size ≥ 40 Mb. Hybridization was carried out at 55°C for 18 hours using 1 µg of each replicate library. Enriched libraries were amplified with 8 cycles of PCR using Post-Capture PCR Oligos and the KAPA HiFi HotStart ReadyMix.

Post-capture Library QC: The quantification of sequencing-ready, post-capture libraries was assessed by qPCR-based method with the KAPA Library Quantification Kit, and the fragment size distribution was analyzed with a 2100 Bioanalyzer instrument and Agilent High Sensitivity DNA Kit (Agilent Technologies).

Library Preparation with the Element Adept™ Library Compatibility Workflow: The target-enriched KAPA EvoPlus and KAPA HyperPlus libraries prepared in the above steps were individually made compatible with the Element AVITI™ System (Element Biosciences). An amount of 0.5 pmol (30 µl of 16.67 nM) of each linear library was circularized using the Element Adept™ Library Compatibility Kit (Element Biosciences). After circularization and cleanup, library concentrations were analyzed by qPCR with the standard and primer mix provided in the Adept™ Kit. The resultant libraries were denatured and sequenced on the Element AVITI™ System.

Data Analysis: Adapters were trimmed during demultiplexing using bcl2fast (v2.20.0.422). The quality trimming was performed using fastp (v0.19.3). Libraries were down-sampled to 60M total raw reads (30M read pairs) and aligned to hg19 reference genome using BWA MEM v0.7.17. Data were further processed according to the technical note How to Evaluate NimbleGen SeqCap EZ Target Enrichment Data.[10]

DeepVariant v0.9.0 was used for variant calling of single nucleotide variants (SNVs) and short insertions and deletions (indels); hap.py v0.3.14 was used to benchmark variant calls against the NA12878 truth set at GIAB v3.3.2 according to the technical note How to Evaluate KAPA Target Enrichment Data For Germline Variant Research.[11]

3. Human WGS

Library Preparation with KAPA HyperPrep Kit: 1 µg of Human genomic DNA from Coriell Institute (NA24385) were sheared to the average 300-350 bp fragment size range by Covaris (ME220). Triplicate libraries were prepared with the KAPA HyperPrep Kit PCR-free workflow. Indexing was incorporated during the adapter ligation step with KAPA Unique Dual-Index Adapters.

Application	Total reads (M)	% of Reads Passing Filters	% Index Assignment	% Q30 or above
Microbial WGS	1089	98.4	96.7	86.4
Human WES	1087	98.7	96.9	86.1
Human WGS PCR-free	1022	98.5	96.8	87.0
Human WGS PCR+	1296	97.5	n/a	88.3

Table 1. Sequencing statistics

Library Preparation with KAPA HyperPlus Kit: Another set of triplicate libraries was prepared with the KAPA HyperPlus Kit and 100 ng gDNA input (NA12878), using enzymatic DNA fragmentation for 10 min at 37ºC. Ligation was performed with 5 µl of 15 µM of KAPA Unique Dual-Index Adapters, and followed by 0.5X/0.66X bead clean-up, and 5 cycles of PCR amplification using KAPA Library Amplification Primer Mix.

Final Library QC: All libraries were quantified with the qPCR-based KAPA Library Quantification Kit for Illumina platforms. Library size distributions were confirmed with a 2100 Bioanalyzer instrument and Agilent High Sensitivity DNA Kit.

Library Preparation with the Element Adept™ Library Compatibility Workflow: The KAPA HyperPrep and KAPA HyperPlus libraries prepared in the above steps were individually made compatible with the Element AVITI™ System (Element Biosciences). An amount of 0.5 pmol (30 µl of 16.67 nM) of each linear library was circularized using the Element Adept™ Library Compatibility Kit (Element Biosciences). After circularization and cleanup, library concentrations were analyzed by qPCR with the standard and primer mix provided in the Adept™ Kit. The resultant libraries were denatured and sequenced on the Element AVITI™ System.

Data analysis: Analysis was performed using FASTQ files with basecalls and quality scores as an input into the DNAScope analysis pipeline from Sentieon using a publicly available platform specific model. Human builds comprised of 360 million 2x150 paired end reads resulted in 35X coverage. After alignment to the hg38 reference and variant calling, the variant calls were evaluated following best practices for benchmarking small variants with the new v4.2.1 GIAB benchmark sets and genome stratifications.[12]

Results and Discussion

1. Sequencing QC metrics

Sequencing was performed using the Element AVITI™ sequencing system. Run quality metrics were reported in real time and at the conclusion of the run using a 1% PhiX library spike-in. All sequencing runs achieved greater than 800 million reads and exceeded specifications of 85% Q30 or greater. **Table 1** shows total number of reads, % passing filters, % index assignment, and % Q30 metrics for each respective application. Human WGS runs were repeated in triplicate, and representative data from a single run was reported. Index assignment was reported only for runs that contained multiplexed samples and required dual-index reads.

2. Microbial WGS

GC-bias is a potential artifact in Next Generation Sequencing that presents as uneven coverage of GC content in a genome and can greatly reduce the amount of information available for analysis. GC bias is more expected when the GC content coverage of the sample is variable and can be introduced at several steps of the library preparation process, such as fragmentation, adapter addition, library amplification, sequencing, and data analysis.

Figure 2 shows the sequencing coverage across regions of various GC content. The bacterial species *C. difficile* (29% GC), *E.coli* (51% GC) and *B. pertussis* (68% GC) are all relevant for human health and were selected to represent a wide range of genomic GC content. GC-bias for the workflows with KAPA HyperPlus and KAPA EvoPlus Kits was assessed by calculating the normalized coverage across GC content using 100 bp-window bins. Distribution of GC content in the genome is indicated by the gray histograms. In the absence of sequencing bias, all bins would be equally represented, indicated by a horizontal distribution centered on a normalized coverage of 1.

Libraries prepared with both KAPA HyperPlus and KAPA Evoplus Kits performed well across varying GC content, especially with respect to the extremely GC-rich (>70% GC) regions of the *B. pertussis* genome, where a decrease in coverage is expected to occur.

These results demonstrate that libraries prepared with the KAPA EvoPlus and KAPA HyperPlus Kits can be successfully converted and sequenced with the Element AVITI™ System, providing highly uniform coverage data from bacterial WGS.

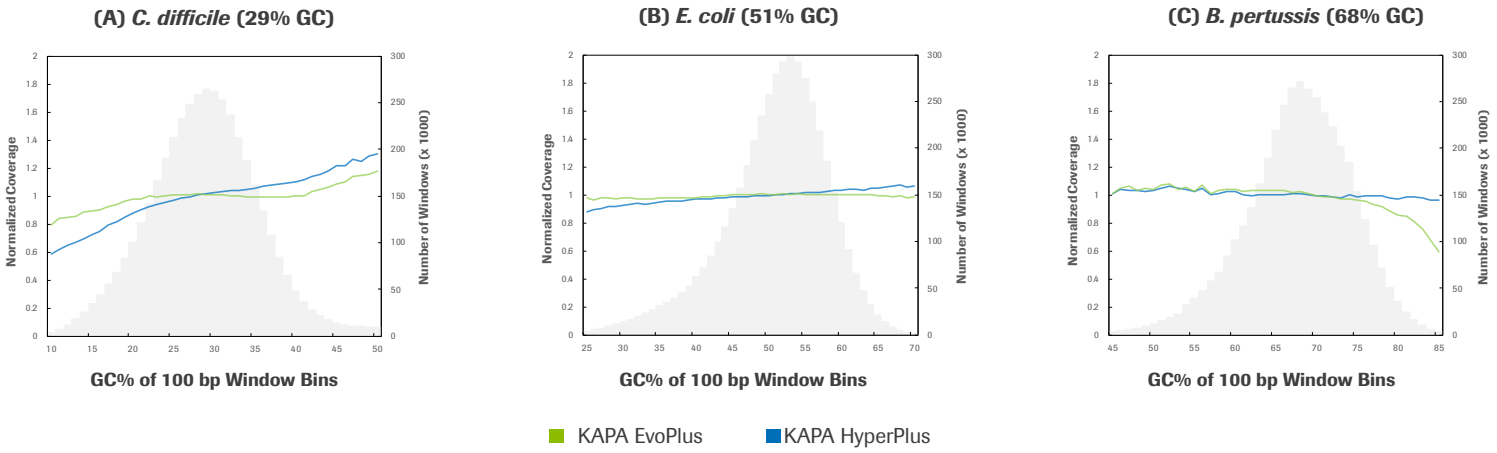

Figure 2. Libraries generated with KAPA EvoPlus and KAPA HyperPlus Kits and sequenced on the Element AVITI™ System showed high uniformity for bacterial species with a wide range of genomic GC content. GC-bias plots were generated with Picard CollectGCBiasMetrics. Gray histograms represent the distribution of genomic GC content of each bacterium, calculated for the reference sequence in 100 bp bins. GC-bias for each workflow was assessed by plotting the normalized coverage for each bin, as average of three replicate libraries. If all sample-to-data processes were completely unbiased, all bins would be equally represented and the plot for each workflow would be a horizontal distribution centered on a normalized coverage of 1.

3. Human WES

The performance of KAPA EvoPlus and KAPA HyperPlus libraries prepared from 100 ng of human genomic DNA input and sequenced on the Element AVITI™ System was evaluated by assessing key sequencing metrics **(Figure 3)** and variant-calling results **(Table 2)**.

Data was down-sampled to 60M total reads prior to analysis, and the resulting mean coverage over the entire target region was similar between workflows **(Figure 3A)**. The coverage of target bases at depths ≥10X and ≥20X was above 97% and 95%, respectively **(Figure 3E)**.

Both workflows using KAPA EvoPlus and KAPA HyperPlus Kits yielded highly efficient libraries. The percent of on-target reads—defined as the proportion of mapped, non-duplicated reads that align to the target region by at least 1 base—was above 80% **(Figure 3B)**. The coverage uniformity—expressed here as fold-80 base penalty—was below 1.5 **(Figure 3C)**, which is within the expected range for the KAPA HyperCap workflow v3 with the KAPA HyperExome probes. The fold-80 base penalty is defined as the amount of additional sequencing required to achieve the mean coverage level for 80% of the sequenced bases. A lower value indicates higher uniformity, and a hypothetical case of perfect uniformity would have a fold-80 base penalty of 1.0.

Another metric for uniformity is the GC content bias, which describes the dependence between the read coverage and the GC content of the region of interest. When all regions are equally represented regardless of GC content, the normalized coverage across all regions is 1.0; when GC-poor and/or GC-rich regions are under- or over- represented in the sequencing data, the normalized coverage is greater or less than 1.0. Both workflows tested in this study showed high uniformity across the GC% spectrum of the KAPA HyperExome panel **(Figure 3F)**.

Library Preparation	# Replicates	Recall SNV	Precision SNV	F1 SNV	Recall INDEL	Precision INDEL	F1 INDEL
KAPA EvoPlus Kit	3	0.9939	0.9945	0.9942	0.9460	0.9335	0.9460
KAPA HyperPlus Kit	3	0.9946	0.9950	0.9948	0.9462	0.9337	0.9399

Table 2. KAPA EvoPlus Kit and KAPA HyperPlus Kit combined with the Element AVITI™ System for human WES enabled variant detection with high sensitivity and precision. DeepVariant and hap.py were used for SNVs and INDELs variant calling, with NA12878 as the truth set. Recall, precision and F1-score values represent the mean of triplicate libraries for each workflow. Recall or sensitivity is the ability to detect variants that are known to be present. Precision or specificity is the ability to correctly identify the absence of variants. F1-score is determined by the harmonic mean of precision and recall.

The level of PCR duplicates in sequencing data indicates library complexity, with low values indicating greater complexity and fewer wasted sequencing reads. Both KAPA HyperPlus and KAPA EvoPlus workflows yielded high-complexity libraries, with a duplication rate of 1.55%, on average, for KAPA EvoPlus Kits and 0.67% for KAPA HyperPlus Kits **(Figure 3D)**. The final yields of libraries generated with the KAPA EvoPlus Kit were slightly lower than KAPA HyperPlus Kit (data not shown), likely leading to the difference in duplication rate between both kits.

Standard human genomic DNA (NA12878) containing validated variants was used in this study in order to determine the variant-calling performance for the two most commonly assessed germline variant classes, namely single nucleotide variants (SNVs) and small insertions and deletions (indels). SNVs were detected with a high degree of sensitivity—denoted here as recall—and precision (>0.99) for both workflows with KAPA EvoPlus and KAPA HyperPlus Kits; indels were called with sensitivity and precision of >0.94 and >0.93 on average, respectively. Detailed results are given in **Table 2**.

Together, these results demonstrate that the KAPA EvoPlus and KAPA HyperPlus Kits are compatible with sequencing on the Element AVITI™ System, generating very uniform and complex libraries that enable variant detection with high sensitivity and precision.

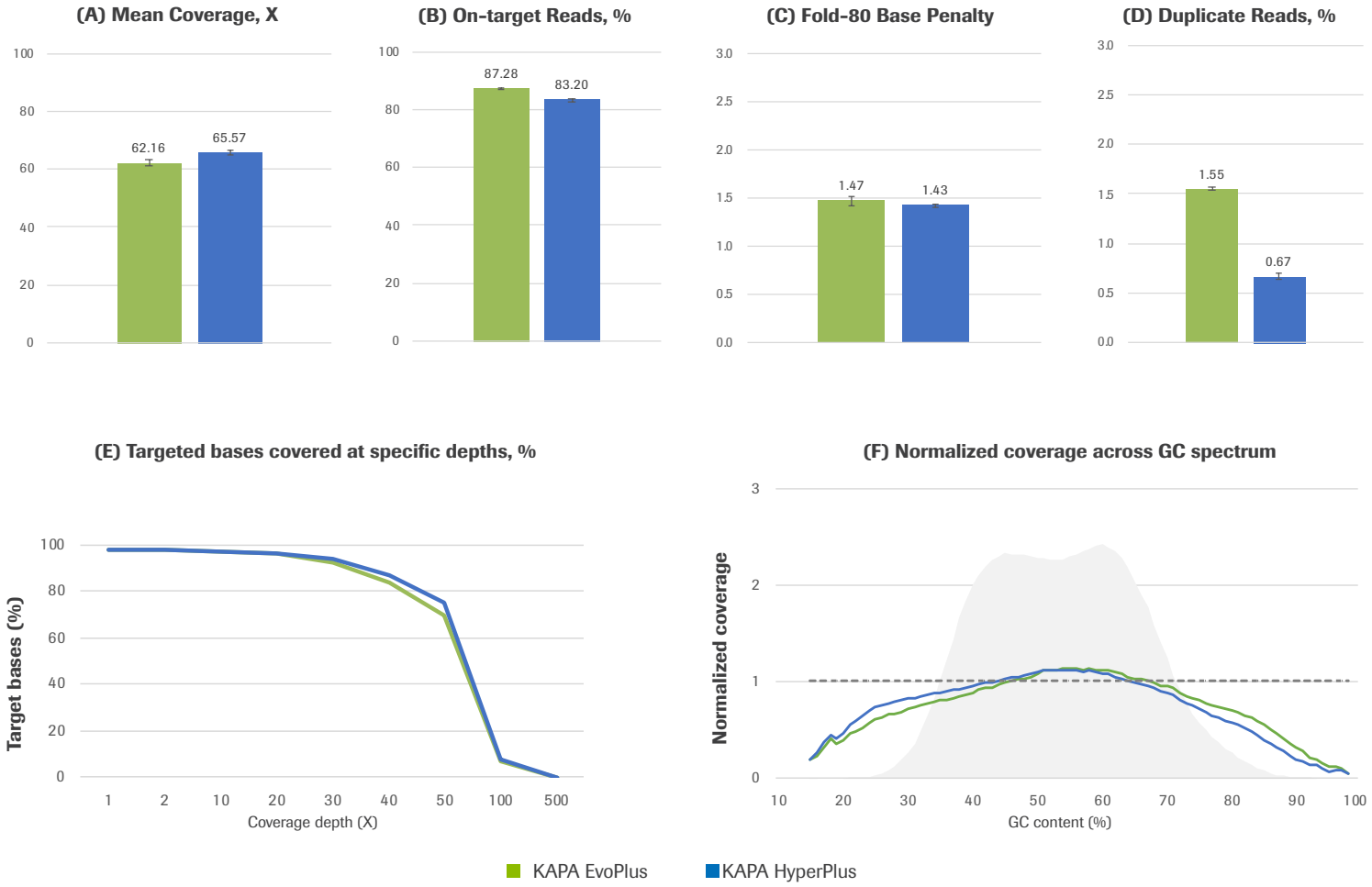

Figure 3. Libraries generated with the KAPA HyperCap v3.0 workflow and sequenced on the Element AVITI™ System yielded high-quality sequencing data for human WES. (A) Mean depth of coverage. **(B)** The percent of on-target reads refers to the percent of mapped, non-duplicate reads overlapping a target region by at least 1 base. **(C)** Coverage uniformity is expressed as fold-80 base penalty. **(D)** Percent of duplicate reads, associated to the library complexity. **(E)** Target enrichment efficiency visualized as the percent of targeted bases covered at specific depths. **(F)** Normalized coverage across the GC spectrum. Each curve represents the average of normalized coverage across GC% bins for triplicate libraries. The horizontal line represents the optimal normalized coverage, which would be 1.0 if all sample-to data steps were completely unbiased. The shaded plot is the GC% distribution of 100 bp windows in the target region. Bars and curves represent the average from triplicate libraries, and error bars indicate the standard deviation. Data was downsampled to 60M total raw reads.

Library Preparation	gDNA Input	RunID	Precision INDEL	Recall INDEL	F1 INDEL	Precision SNP	Recall SNP	F1 SNP
KAPA HyperPlus Kit (PCR+)	NA12878	Element 1	0.9886	0.9843	0.9864	0.9977	0.9932	0.9955
		Element 2	0.9905	0.9870	0.9887	0.9979	0.9942	0.9960
		Element 3	0.9882	0.9845	0.9864	0.9977	0.9939	0.9958
KAPA HyperPrep Kit (PCR-free)	NA24385	Element 4	0.9977	0.9926	0.9951	0.9980	0.9910	0.9945
		Element 5	0.9980	0.9933	0.9956	0.9980	0.9912	0.9946
		Element 6	0.9976	0.9923	0.9949	0.9981	0.9921	0.9951

Table 3. KAPA HyperPlus and KAPA HyperPrep Kits combined with the Element AVITI™ System for human WGS enabled variant detection with high sensitivity and precision. Recall, precision and F1-score values represent individual values for each workflow. Recall or sensitivity is the ability to detect variants that are known to be present. Precision or specificity is the ability to correctly identify the absence of variants. F1-score is determined by the harmonic mean of precision and recall.

4. Human WGS

Both SNP and indel calls were highly accurate with F1 scores exceeding 0.995 **(Table 3)**. The library preparation with the KAPA HyperPlus workflow resulted in slightly lower indel precision, recall, and F1 measurements compared to the workflow with the KAPA HyperPrep Kit. SNP metrics were more consistent than indel metrics between workflows.

To determine human genome coverage uniformity, aligned reads were down sampled to 35X and GC-bias plots were generated using Picard CollectGcBiasMetrics. Both KAPA HyperPrep and KAPA HyperPlus libraries sequenced on the Element platform showed strong uniformity of coverage between 20% and 80% GC **(Figure 4)**. As expected, the extreme ends of the GC plot showed greater variation, but only comprise a small fraction of the human genome.

In conclusion, the KAPA HyperPrep and KAPA HyperPlus Kits are compatible with the Element AVITI™ sequencing system, generating unform high quality full human genome builds with high variant calling precision and recall metrics.

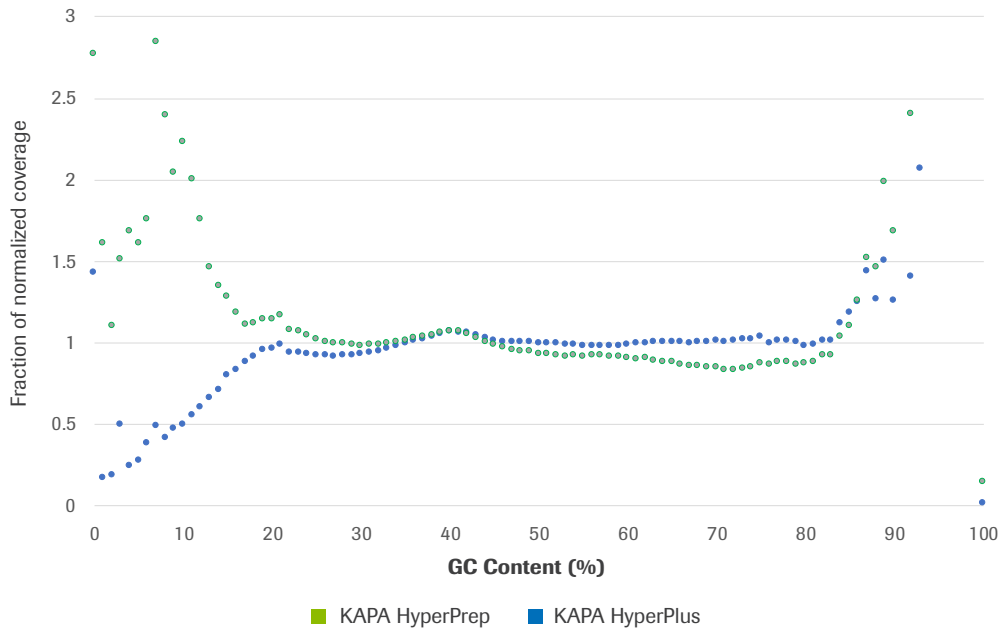

Figure 4. KAPA HyperPrep and KAPA HyperPlus libraries sequenced on the Element AVITI™ System showed strong uniformity of coverage for human WGS. Data was downsampled to 35X and GC bias plots were generated using Picard CollectGcBiasMetrics.

Conclusions

The KAPA Library Prep portfolio, including the KAPA HyperPrep, KAPA HyperPlus, and KAPA EvoPlus, enable high quality library preparation across multiple sequencing platforms, demonstrated here with the new Element AVITI™ system. Libraries prepared using KAPA Library Prep kits are easily converted to be compatible with this system using the Element Adept™ Library Compatibility Kit while maintaining quality across key metrics for a diverse set of applications. Bacterial WGS samples prepared with KAPA HyperPlus and KAPA EvoPlus Kits show high uniformity of coverage across a broad spectrum of genomic GC content post-conversion, highlighting the utility of these methods for a wide range of genomes as input. Additionally, human WGS samples prepared with both mechanical (using KAPA HyperPrep Kit) and enzymatic (using KAPA HyperPlus Kit) fragmentation maintain high quality and sensitive detection of variants post-conversion. Target enrichment using KAPA HyperCap workflow v3.0 following library preparation with both KAPA HyperPlus and KAPA EvoPlus Kits also flows easily into conversion to the Element AVITI™ system, allowing for high quality human WES sequencing on this platform. The robustness of libraries prepared using the KAPA Library Prep portfolio allows for high quality sequencing results across multiple applications and input types following conversion to this new sequencing platform.

References

1. Weimer BC. 2017. 100K Pathogen Genome Project. Genome Announc 5:e00594-2017.
2. Lappalainen et al. Genomic Analysis in the Age of Human Genome Sequencing. Cell 177, March 21, 2019.
3. Suwinski et al. Advancing Personalized Medicine Through the Application of Whole Exome Sequencing and Big Data Analytics. Front. Genet. 10:49, 2019.
4. Roche Sequencing & Life Science. Application Note. Miller B, et al. A novel, single-tube enzymatic fragmentation and library construction method enables fast turnaround times and improved data quality for microbial whole-genome sequencing. December 2017.
5. Roche Sequencing & Life Science. Application Note. Meyer J, et al. KAPA HyperPrep Kits offer a flexible, high-efficiency library preparation solution for PCR-free human whole-genome sequencing. April 2018.
6. Roche Sequencing & Life Science. Technical Note. The KAPA HyperCap Workflow v3 yields high-quality results with hybridization times as short as one hour using mechanical DNA fragmentation. October 2020.
7. Roche Sequencing & Life Science. Application Note. Pavlica J, et al. KAPA HyperPrep: A streamlined solution for the construction of ChIP-Seq libraries from picogram amounts of DNA. March 2019.
8. Roche Sequencing & Life Science. Application Note. Hapshe N, et al. High-efficiency, species-specific ribosomal RNA depletion with the KAPA RNA HyperPrep Kit with RiboErase (HMR). February 2019.
9. Roche Sequencing & Life Science. Application Note. Nabilsi N, et al. SeqCap HyperEpi: Integrating the KAPA HyperPrep Kit and the SeqCap Epi Enrichment System for a robust targeted bisulfite sequencing workflow. June 2018.
10. Roche Sequencing Solutions. Technical Note. How To Evaluate NimbleGen SeqCap EZ Target Enrichment Data. August 2015.
11. Roche Sequencing Solutions. Technical Note. How to Evaluate KAPA Target Enrichment Data For Germline Variant Research. 2021.
12. Krusche P, et al. Best practices for benchmarking germline small-variant calls in human genomes. Nature Biotechnology vol. 37: 555-560. 2019.

For more information about Roche KAPA DNA Library Kits, please visit: **go.roche.com/dnalp**

Published by:
Roche Sequencing and Life Science
9115 Hague Road
Indianapolis, IN 46256
sequencing.roche.com

MC-US-10708 APP100001 A692 3/22