

Streamlined Library Prep Workflow for Element Biosciences AVITI™ Sequencer using sparQ DNA Frag & Library Prep Kit from Quantabio

Keywords: sparQ DNA Frag & Library Prep Kit, Element Biosciences AVITI™ sequencing system, sparQ PureMag Beads, PCR-free library preparation

ABSTRACT

Avidite Base Chemistry™ (ABC™) sequencing technology brought a significant advancement to next-generation sequencing (NGS). It is unique to Element Biosciences and powers the AVITI™ sequencing systems. This application note outlines the DNA library preparation process and the necessary protocol adjustments when using the sparQ DNA Frag & Library Prep Kit for the AVITI™ sequencing platforms. Developed in collaboration with Element Biosciences, these modifications enable the generation of high-quality libraries and sequencing data.

Introduction

Next-generation sequencing (NGS) has greatly advanced genomic research by enabling high-resolution analysis of genetic information. Short-read sequencing remains the dominant technology in the field, widely adopted by genomics and translational research laboratories. As the demand for sequencing continues to grow, there is an increasing need for platforms that offer improved accuracy, efficiency, and cost-effectiveness.

The AVITI™ benchtop sequencing platform by Element Biosciences utilizes a novel Avidite Base Chemistry™, enabling high-fidelity base calling. This method relies on the use of avidites, which are fluorescently-labeled, multivalent nucleotide polymers designed to bind DNA templates with high specificity and low dissociation rates¹. By separating signal generation from nucleotide incorporation, avidity sequencing permits the use of unlabeled nucleotides during extension steps. This reduces incorporation-associated artifacts and enhances overall sequencing accuracy.

To improve the sequencing workflows and streamline the upstream processes, Quantabio, in collaboration with Element Biosciences™, has developed an optimized library preparation workflow for whole-genome sequencing (Figure 1). This workflow leverages the sparQ DNA Frag & Library Prep Kit, a reliable and efficient solution for DNA library preparation.

This application note describes the use of the sparQ DNA Frag & Library Prep Kit to prepare libraries for sequencing on the Element AVITI™ System, a protocol that utilizes the Elevate™ Long UDI Adapters for PCR-free and xGen® Stubby Adapter-UDI Primers (IDT) for amplified library preparation. This streamlined Quantabio NGS workflow provides an efficient and accessible solution for laboratories seeking to adopt the AVITI™ platform while achieving high-quality sequencing data with improved accuracy and efficiency.

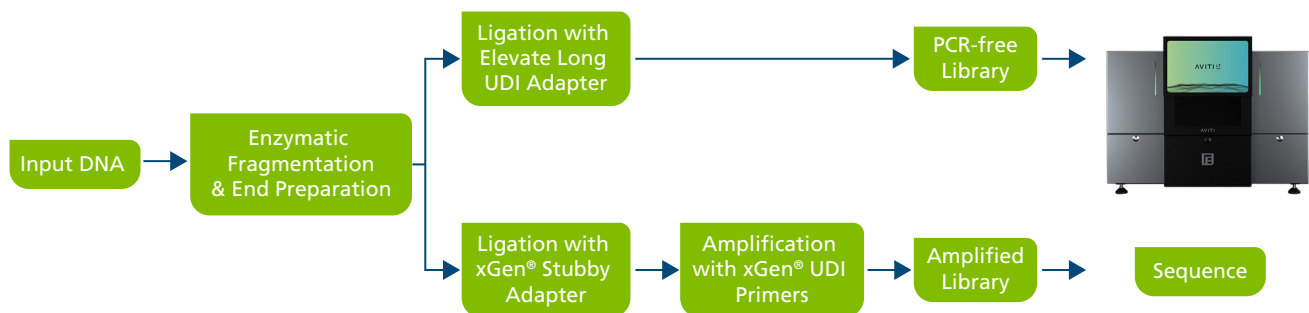


Figure 1 Workflow for library preparation using the sparQ DNA Frag & Library Prep Kit for sequencing on the Element AVITI™ System. The workflow supports PCR-free library preparation with the Element Biosciences Elevate™ Long UDI Adapter, as well as amplified library preparation with the IDT xGen® Stubby Adapter-UDI Primers.

Methods and Materials

DNA Samples

High molecular weight human genomic DNA (Cat. No. 11691112001, Roche) without additional purification and mixed bacterial genomic DNA (1:1:1 ratio of *Fusobacterium nucleatum*, *Escherichia coli*, and *Bordetella pertussis*, sourced from ATCC) were used in this study. The protocol was optimized for input amounts of 10 ng, 100 ng, and 500 ng of human genomic (HG) DNA, as well as 200 ng of mixed bacterial (MB) genomic DNA.

Library Preparation and Sequencing

PCR-free libraries were prepared using 200 ng of MB genomic DNA or 500 ng of HG DNA as input with the sparQ DNA Frag & Library Prep Kit. For both sample types, fragmentation was carried out by incubating with the DNA Frag & Polishing Master Mix at 32 °C for 4 minutes, following the protocol described in the product manual (IFU-144.1 REV 01 95194 sparQ-DNA-Frag-Library-Prep-Kit-Element-0825). Elevate™ Long UDI Adapter Kit Set A (830-00010, Element Biosciences) were used at a stock concentration of 15 µM, with 4 µl added for 500 ng of input DNA while a 1:2 dilution was used for 200 ng of input DNA. PCR-free libraries were purified with sparQ PureMag Beads and eluted in 10 µL of elution buffer (10 mM Tris-HCl, pH 8.0).

Amplified libraries were prepared from 10 ng, 100 ng, and 500 ng of HG DNA as input. Samples were fragmented using the DNA Frag & Polishing Master Mix at 32 °C for 8, 5, and 4 minutes, respectively. A volume of 4 µl of xGen® Stubby Adapter for Element (IDT Catalog #10017037) at 15 µM was added to the fragmented DNA in the presence of the Ligation Master Mix and incubated at 20 °C for 15 minutes. Following ligation, 5 µl of xGen® UDI Primers Element was added for PCR amplification using either the sparQ HiFi PCR Master Mix (a component of the sparQ DNA Frag & Library Prep Kit) or repliQa HiFi ToughMix following the 3-step cycling protocol with 1 second for the extension step. Libraries from inputs of 10 ng, 100 ng, and 500 ng of HG DNA were amplified for 9, 5, and 3 PCR cycles, respectively. Finally, the amplified libraries were purified using sparQ PureMag Beads and eluted in 30 µL of elution buffer (10 mM Tris-HCl, pH 8.0).

Size Verification and Quantification

To assess the size distribution of the PCR-free libraries, 1 µl of the libraries was amplified using HiFi PCR Master Mix (2X) and xGen® Library Amp Primer Mix for Element (IDT Catalog #10016959) before using TapeStation. The size of the libraries was assessed using a D5000 ScreenTape on the 4200 TapeStation® System (Agilent). Library concentrations were

determined with the Qubit® dsDNA HS Assay Kit (Thermo Fisher Scientific) using a Qubit® 3.0 Fluorometer, following the manufacturer's instructions.

Pooled libraries were sequenced using Cloudbreak Freestyle™ chemistry with paired-end 2x151 cycle lengths, generating 945.6M paired-end reads at 96.8% Q30. FASTQ files were processed using bases2fastq version 2.0.0 with default adapter trimming settings (--settings R1AdapterTrim, True --settings R2AdapterTrim, True).

Results

In collaboration with Element Biosciences, we optimized PCR-free and PCR-amplified library preparation workflows using the sparQ DNA Frag & Library Prep Kit for compatibility with the AVITI sequencing platform. These library prep protocols were evaluated using human genomic DNA and mixed bacterial genomic DNA.

PCR-free library preparation offers advantages in whole genome sequencing (WGS) by eliminating amplification bias, leading to more uniform coverage and higher variant detection accuracy, especially for difficult-to-sequence GC-rich regions. It also reduces library duplication rates, leading to better data utilization and potentially lower sequencing costs. In this workflow, Elevate™ Long UDI Adapters were used and incorporated during the ligation step to enable the generation of PCR-free libraries. Typically, higher input amounts are required for PCR-free library preparation. In this application note, we used 500 ng of human genomic (HG) DNA and 200 ng of mixed bacterial genomic (MB) which represents a broad range of GC content: *F. nucleatum* (27% GC), *E. coli* (50% GC), and *B. pertussis* (67% GC). This design allows us to assess potential biases introduced during the fragmentation step across varying GC-content. PCR-free libraries were analyzed on the TapeStation after 5 cycles of amplification. Both HG and MB PCR-free libraries displayed the expected migration profiles, with an average library size of ~800 and ~600 bp, respectively (Figure 2A).

Library yields were quantified using Qubit®, showing that an average of 350 ng of libraries was generated from 500 ng of HG DNA, while the libraries prepared from 200 ng of MB DNA produced 170 ng library (Figure 2B). These concentrations exceed the minimum requirements for sequencing, demonstrating that the sparQ workflow consistently delivers PCR-free libraries with uniform fragment profiles and sufficient yield across different DNA sample types.

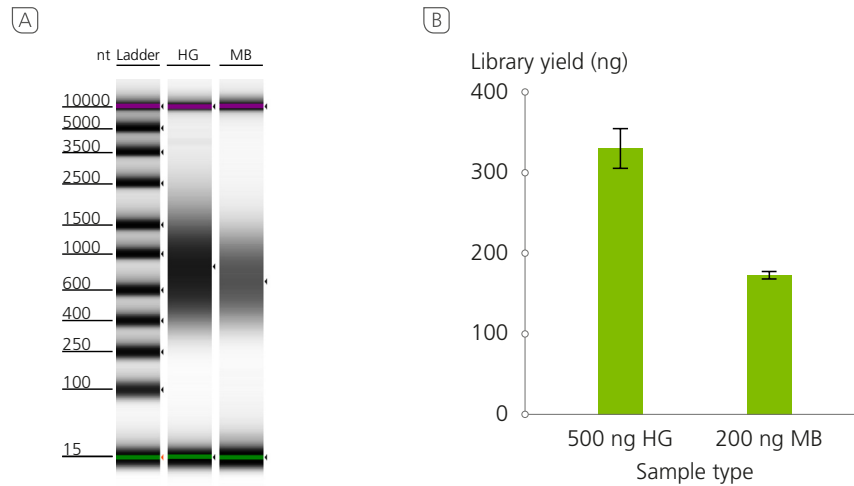


Figure 2 PCR-free libraries from different DNA samples using Elevate™ Long UDI Adapters. A Electropherogram of PCR-free libraries run on a D5000 ScreenTape™ on the Agilent 4200 TapeStation™. B Total PCR-free library yield generated from the library preparation with sparQ DNA Frag & Library Prep Kit and Elevate™ Long UDI Adapters.

PCR-free libraries were sequenced on the Element AVITI™ System. The performance of the sparQ DNA Frag & Library Prep Kit for PCR-free library generation was evaluated using key sequencing metrics, which includes number of reads, duplication rates, and mapped reads percentage.

A total of 50 Gb reads were generated from HG DNA, and 22 Gb reads from MB genomic DNA libraries. Both sample types exhibited low duplication rates (4.1% for HG and 11.6% for MB) and high percentages of mapped reads (98.6% for HG and 87.6% for MB libraries) (Table 1). In addition, HG and MB libraries achieved PHRED scores above 40, reflecting excellent base-calling accuracy and overall sequencing performance (Figure 3A).

Normalized coverage analysis of the 1:1:1 bacterial genomic DNA mixture further demonstrated uniform sequencing coverage across a broad GC range (10% to 85%) (Figure 3B). This result indicates minimal GC bias and highlights the capability of the sequencing chemistry to accurately capture genomic regions with varying GC content. Together, these metrics emphasize the robustness of the sparQ DNA Frag & Library Prep Kit in combination with Elevate™ Long UDI Adapters for PCR-free library preparation, enabling the generation of high-quality libraries with minimal bias across different genome compositions and sample types.

	PCR-free Workflow		PCR-amplified Workflow	
	HG (500 ng)	MB (200 ng)	HGS (100 ng)	HGR (100 ng)
Input DNA	HG (500 ng)	MB (200 ng)	HGS (100 ng)	HGR (100 ng)
Duplication rate (%)	4.1 (± 0.32)	11.6 (± 5)	3.1 (± 0.2)	3 (± 0.1)
Total mapped reads	509,151,113 (± 43,357,031)	21,932,864 (± 1,084,485)	288,894,106 (± 11,454,219)	342,457,851 (± 8,833,836)
Mapped reads (%)	98.6 (± 0.3)	87.6 (± 1.3)	99.6 (± 0.1)	99.7 (± 0.1)
Reads mapped in pairs (%)	83 (± 0.7)	83.3 (± 0.9)	88 (± 0.1)	92.5 (± 0.2)
Average coverage	25 (± 2)		14 (± 1)	16

Table 1 High-quality sequencing metrics for PCR-free and amplified libraries. Sequencing metrics are shown for PCR-free libraries from 500 ng human genomic (HG) DNA and 200 ng mixed bacterial (MB) genomic DNA, and for amplified libraries prepared from 100 ng HG DNA using sparQ HiFi PCR Master Mix (HGS) or repliQa HiFi ToughMix (HGR). Results represent mean values and standard deviation.

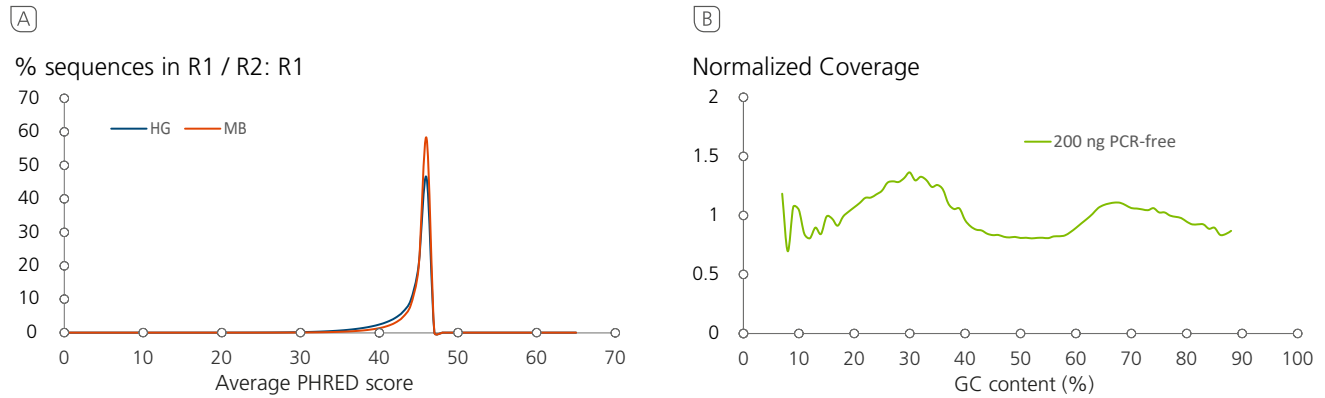


Figure 3 Sequencing metrics of PCR-free library preparation using Elevate™ Long UDI Adapter. **A** Quality distribution of sequencing data of human genomic DNA (HG, blue) and mixed bacterial genomic DNA (MB, orange) based on the percentage of sequences in R1/R2 with a given PHRED score. **B** Normalized coverage as a function of GC content for the sample mixed bacterial genomic DNA using the PCR-free workflow.

PCR-free workflows are advantageous for whole-genome sequencing because it eliminates amplification-associated bias; however, it requires a high amount of input DNA, which may not always be available. In applications where the input DNA samples are of low quantity or quality (e.g. FFPE samples), or shotgun metagenomic studies from samples with very little starting DNA, PCR amplification is necessary to generate enough material for sequencing. Additionally, applications involving hybrid capture require a higher library yield as an input for the hybrid capture step. To address these needs, a PCR-amplified library preparation workflow is essential. Thus, we optimized an amplified workflow using the sparQ DNA Frag & Library Prep Kit in combination with xGen® Stubby Adapters (IDT). Libraries were prepared from

10 ng, 100 ng, and 500 ng of HG DNA with fragmentation times of 8, 5, and 4 minutes, respectively.

The resulting libraries displayed the expected migration profile, with an average library size of 645 bp (500 ng), 590 bp (100 ng), and 540 bp (10 ng) (Figure 4A). Library yields quantified using Qubit® showed that inputs of 500 ng, 100 ng, and 10 ng PCR amplified with 3, 5, and 9 PCR cycles produced 1275 ng, 570 ng, and 870 ng of total library, respectively (Figure 4B). This protocol demonstrated that PCR amplified libraries can be achieved with high yield, even when starting from lower DNA input, expanding the flexibility of the sparQ DNA Frag & Library Prep Kit to accommodate a wide range of sample types and input amounts.

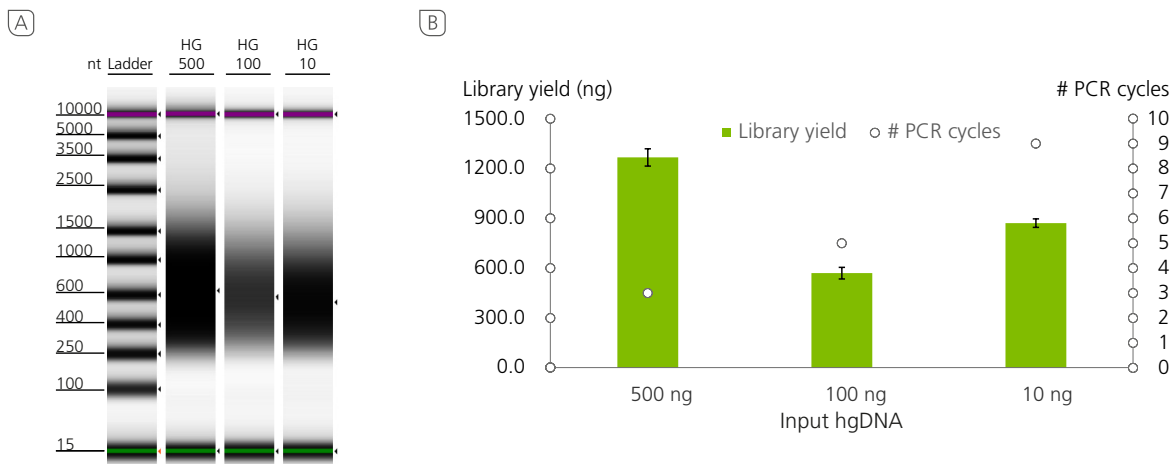


Figure 4 Amplified library preparation using xGen® Stubby Adapter. **A** Electropherogram of amplified libraries run on the Agilent 4200 TapeStation™ using a D5000 ScreenTape™. **B** Total library yield generated from 500 ng, 100 ng, and 10 ng of HG DNA, which required 3, 5, and 9 PCR cycles.

The polymerase used for library amplification plays an essential role, as differences in fidelity and amplification bias directly affect the accuracy, reliability, and representation of sequencing data. We prepared libraries from 100 ng of HG DNA and amplified them using two different master mixes. The first was sparQ HiFi PCR Master Mix, included in the sparQ DNA Frag & Library Prep Kit, which has consistently demonstrated robustness over the years. The second was repliQa HiFi ToughMix, demonstrated as the best PCR enzyme for library amplification in NGS by the researchers from the Wellcome Sanger Institute, mainly due to low GC bias, uniform amplification across different DNA templates,

and reduced error rates². Libraries prepared with both master mixes showed comparable size distributions, with an average library size of 570 bp (Figure 5A). Library yields measured by Qubit[®] were also similar, with sparQ-prepared samples producing 600 ng and repliQa-prepared samples producing 560 ng library (Figure 5B). Furthermore, sequencing metrics were nearly identical between the two master mixes, with both generating ~30 Gb of total reads, low duplication rates (~3%) and high percentage of mapped reads (>99%) (Table 1). These results demonstrate that both high-fidelity master mixes support efficient library amplification with consistent size profiles, yields and sequencing metrics.

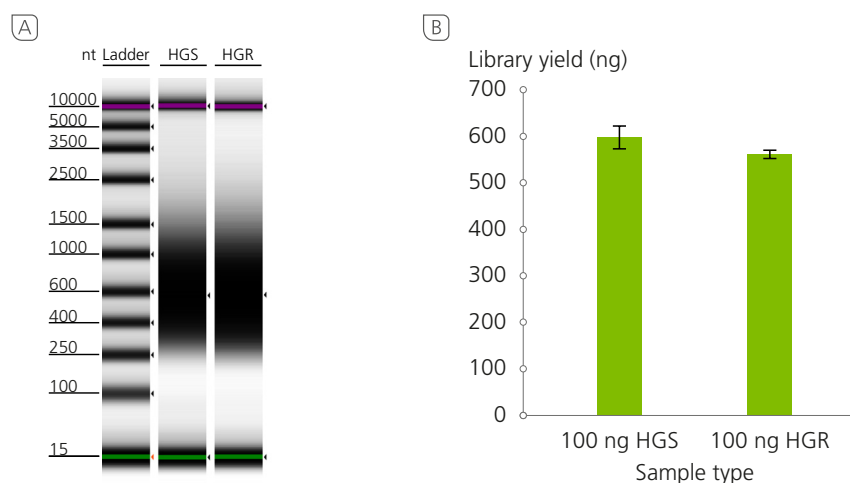


Figure 5 Library amplification using different HiFi polymerases. **A** Electropherogram of amplified libraries from 100 ng of human genomic DNA using sparQ HiFi PCR Master Mix (HGS) or repliQa HiFi ToughMix (HGR) run on the Agilent 4200 TapeStation™ using a D5000 ScreenTape™. **B** Total library yields as measured by Qubit[®] quantification.

Conclusions

In this application note, we demonstrated that the sparQ DNA Frag & Library Prep Kit, in combination with sequencing on the Element Biosciences AVITI™ platform, generated high-quality libraries for both PCR-free and amplified workflows. This flexibility provides researchers with a versatile library preparation strategy that accommodates a wide range of sample types and input amounts. These workflows can support the adoption of the AVITI™ system in genomics laboratories, providing reliable and streamlined library preparation solutions.

Reference

1. Sinan Arslan, Francisco J. Garcia, Minghao Guo, Matthew W. Kellinger *et al.* Sequencing by avidity enables high accuracy with low reagent consumption. *Nature Biotechnology* 42, 132–138 (2024). <https://doi.org/10.1038/s41587-023-01750-7>
2. Michael A. Quail, Craig Corton, James Uphill, Jacqueline Keane, Yong Gu. Identifying the best PCR enzyme for library amplification in NGS. *Microbial Genomics* 2024 Apr;10(4):001228. doi: 10.1099/mgen.0.001228.

Trademarks: Element AVITI™ System, Element Elevate™ workflow, Cloudbreak Freestyle™, Avidite Base Chemistry™ and ABC™ are trademarks of Element Biosciences, Inc. xGen® is a registered trademark of Integrated DNA Technologies, Inc. repliQa® and ToughMix® are registered trademarks of Quantabio. ScreenTape® and TapeStation® are registered trademarks of Agilent Technologies, Inc. Qubit® is a registered trademark of Thermo Fisher Scientific. Quantabio products are intended for molecular biology applications. The products are not intended for the diagnosis, prevention or treatment of a disease.

MK-AN-0028 REV 01 Streamlined Library Prep Workflow for Element Biosciences AVITI Sequencer 0226