High Compatibility for RNA Sequencing Using the Element AVITI System Sequencing Instrument and the Bio-Rad SEQuoia™ Library Prep Workflow

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Abstract

The shift toward alternative sequencing platforms offers cost savings and expands technological options, enhancing sequencing flexibility and advancing scientific applications. The Element AVITI System Sequencing Instrument provides cost-effective RNA sequencing (RNA-Seq) with independent run control, capable of delivering up to 2 billion reads per run. As existing RNA-Seq library prep kits are designed for Illumina® platforms, evaluating compatibility with the use of AVITI Adapters for sequencing is crucial due to its different sequencing chemistry. This study assessed the compatibility of the SEQuoia Complete Stranded RNA Library Prep Kit (Bio-Rad Laboratories, Inc., catalog #17005726) and the SEQuoia Express Stranded RNA Library Prep Kit (Bio-Rad, #12017297) with the Element AVITI System and the corresponding AVITI Adapters, which demonstrate highly accurate RNA-Seq results. The results of this study establish the Element AVITI Sequencing platform as a viable option for RNA-Seq applications using the SEQuoia portfolio of RNA-Seq library preparation kits.

Introduction

The demand for high-resolution RNA-Seq technology is widespread across various applications, from differential gene expression analysis to RNA genome detection (Lowe R et al. 2017). Notably, RNA sequencing for gene expression analysis has been incorporated into clinical trials to identify markers predicting response to immunotherapy (Tsimberidou AM et al. 2022). However, its broader adoption is hindered by its high cost, complex procedures, and the lack of customizable sample preparation workflows for diverse sample types, batch sizes, and sequencing conditions.

Integrating low-quality and low-yield sample types into library preparation can enhance accessibility to common archival specimens like formalin-fixed tissues, as well as rare cells and single-cell isolation (Palomares et al. 2019, Sarantopoulou et al. 2019). Additionally, addressing bias in commercial RNA-Seq library protocols is crucial for detecting both long (>200 bp) and short (<200 bp) transcripts effectively. Otherwise, researchers must typically choose to focus on either long (>200 bp) or short (<200 bp) RNAs. Finally, simpler RNA-Seq library preparation workflows that maintain robust data for higher-quality samples are also needed, thus a rapid RNA library preparation workflow offering shorter time to results is very important.

To address unbiased RNA transcript capture and enhance usability of low-quality and/or low-yield RNA, a prior study (Perike S et al. 2022) with the SEQuoia Complete Stranded RNA Library Prep Kit showed that its unique combination of enzymes can capture both long and short RNA biotypes in a single library. Additionally, combining this kit with the SEQuoia RiboDepletion Kit for rRNA-derived fragment depletion produces comprehensive libraries from formalin-fixed paraffin-embedded (FFPE) RNA comprised of both long and short RNA biotypes, and therefore better represents the complete transcriptome (Perike S et al. 2022).

For projects requiring faster library preparation when targeting only long RNA biotypes in high-quality samples, the SEQuoia Express Stranded Library Prep Kit provides a streamlined three-tube workflow that can be completed in <3 hours with minimal pipetting and hands-on time. Integrated bioinformatics solutions support data analysis for both kits.

BIO-RAD

Table 1 outlines the specifications and unique features of the SEQuoia Complete and SEQuoia Express Stranded RNA Library Prep Kits, detailing RNA subtypes captured, compatible sample types, and protocol differences.

Table 1. Comparison of SEQuoia RNA-Seq Library Prep Kits.

	SEQuoia Complete Stranded RNA Library Prep Kit	SEQuoia Express Stranded RNA Library Prep Kit
Capture range	All RNA subtypes >20 bp	Long RNA fragments >200 bp
Input range	100 pg–1 µg	1 ng–1 µg
Sample type	All sample types, including low-quality FFPE samples	Standard good-quality sample
Sequencing read	Single-read	Paired-end
Protocol time	4 hr	3 hr
RNA subtypes captured	mRNA, lncRNA, miRNA, snoRNA, tRNA, and more	mRNA, lncRNA
Tubes per kit	7	3

FFPE, formalin-fixed paraffin-embedded; lncRNA, long noncoding RNA; snoRNA, small nucleolar RNA

The Element AVITI platform has been shown to have flow cell flexibility for scaling sequencing as needed and reduces the cost of obtaining sequencing results for a broad range of applications. Figure 1 provides an overview of the platform workflow.

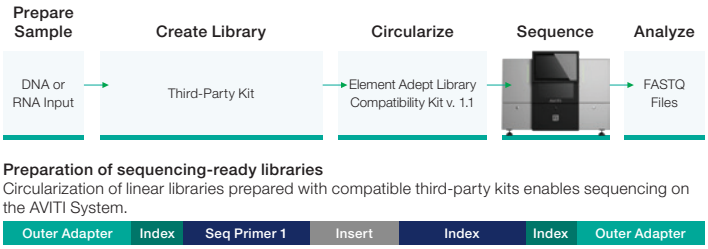


Fig. 1. Element AVITI Sequencing Platform. The Adept Workflow starts with DNA or RNA input prepared with a third-party kit, then circularizes the library for sequencing on the AVITI System. After sequencing, Base2Fastq Software converts the base files into FASTQ files.

The utility of new RNA library preparation reagents that allow rapid and comprehensive transcriptome profiling has not been fully explored with the AVITI platform. This application study assessed the AVITI System in conjunction with both the SEQuoia Express and Complete Stranded Library Prep Kits for the ability to generate high-quality, accurate gene expression data. Specifically, this includes profiling novel transcripts, quantifying long noncoding RNAs (lncRNAs), and analyzing small RNAs.

Our findings highlight the seamless compatibility of both the SEQuoia Express and Complete Stranded RNA Library Prep Kits with the Element AVITI platform, underscoring AVITI as a robust alternative for RNA-Seq studies and the ability of this novel RNA-Seq library portfolio to identify novel transcripts and provide more complete transcriptome profiling.

Materials and Methods

RNA-Seq Workflow

To evaluate the impact of RNA quality and input levels, as well as different sequencing protocols, we designed experimental workflows (Figure 2) using the SEQuoia Express and Complete Stranded RNA Library Prep Kits. The workflows are similar except for the polyadenylation step in the SEQuoia Complete protocol.

Libraries prepared with the SEQuoia Express Kit used human reference RNA (UHR) (Quantitative PCR Human Reference Total RNA, Agilent Technologies Inc., #750500) to assess high-quality RNA input and detect long RNAs, while those prepared with the SEQuoia Complete Kit used Human Placenta Total RNA (Thermo Fisher Scientific Inc., #AM7950), which contains a higher number of micro RNAs (miRNAs) for evaluation than UHR and can be used to evaluate the detection of both miRNAs and long RNAs. For both kits, sequencing results were also evaluated at both low (10 ng) and high (100 ng) RNA inputs to determine any impacts on sequencing performance. Libraries were prepared according to manufacturer instructions using SEQuoia Dual Indexed Primers (Bio-Rad, #12011930). Library quantification was performed with the Qubit 4 Fluorometer (Thermo Fisher Scientific, #Q33238) and analyzed using the 2100 Bioanalyzer Instrument (Agilent).

For ribosomal RNA depletion, 5 ng of complementary DNA (cDNA) from each library was processed using the SEQuoia RiboDepletion Kit (Bio-Rad, #17006487) following manufacturer instructions up until the final amplification or SPRI cleanup step. All libraries were converted to Element-compatible libraries using the Element Adept Library Compatibility Kit v. 1.1 (Element, #830-00007) prior to sequencing.

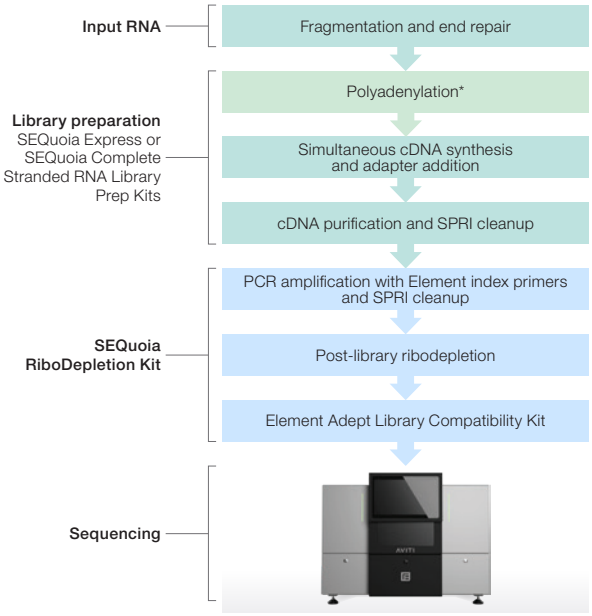
SEQuoia Complete libraries were sequenced on the AVITI System with paired or single-end reads using a 75 × 8 bp configuration (R1 = 75 bp, R2 = 8 bp; or R1 = 75 bp for single-end reads). SEQuoia Express libraries were sequenced on the AVITI System with paired-end reads using a 2 × 75 bp configuration.

Data Analysis

FASTQ files were analyzed using SeqSense NGS Data Analysis Software. The SEQuoia Complete Analysis Toolkit was used with the SEQuoia Complete Kit–generated libraries to generate transcriptome profiling data, while the SEQuoia Express Analysis Toolkit was used for the SEQuoia Express Kit–generated libraries.

By downsampling to 10 million reads, the study compared results by analyzing sample replicates and further analyzed long transcripts (including lncRNAs and protein-coding genes) and miRNAs measured by transcripts per kilobase million (TPM). TPM averages were compared between high (100 ng) and low (10 ng) input RNA across three replicates, along with the number of detected long transcripts and miRNAs with ≥5 reads per transcript or miRNA. Raw RNA-Seq read quality was evaluated using the SEQuoia Toolkit FastQC tool, followed by trimming with the SEQuoia Toolkit CutAdapt tool to remove polyA tails, primers, and adapters from the sequencing contigs. Alignment to the human transcriptome (ENSEMBL Genome Reference Consortium Human Build 38, GRCH38) was performed using the

Experimental Workflow



Element AVITI System

* Only for SEQuoia Complete Library Prep Kit, not the SEQuoia Express Kit.

Fig. 2. RNA-Seq library preparation and sequencing workflow.

SEQuoia Toolkit STAR aligner tool, with Picard tools used for BAM file quality control (QC). Estimated transcript summaries were generated at the gene level, with final reports in PDF, HTML, and CSV file formats.

Results

This study evaluated the compatibility of SEQuoia Express and SEQuoia Complete Stranded RNA Library Prep Kits used with the Element AVITI System Sequencing Instrument across varying RNA input and RNA sources, which differ in terms of RNA subtypes (UHR versus human placental total RNA).

High-quality libraries provided by SEQuoia RNA-Seq library prep kits that are sequenced on the Element AVITI platform are expected to demonstrate robust reproducibility and high sequencing quality, characterized by high Q30 base quality scores and consistent read outputs, at both high and low RNA input levels, as demonstrated in Table 2.

Table 2. Replicate RNA-Seq run metrics using SEQuoia Library Prep Kits on the Element AVITI Sequencer demonstrate excellent sequence quality. Triplicates of the libraries generated by the SEQuoia Complete and SEQuoia Express Kits were constructed for high and low RNA inputs. Sequencing libraries generated using the Element AVITI System achieved high Q30 base quality scores for all replicates.

Metric	SEQuoia Express Kit	SEQuoia Complete Kit
%Q30	95.9	95.8
%Q40	84.3	85.4

For transcript detection, use of the AVITI System with both kits exhibited excellent reproducibility, as illustrated by high correlations in transcript counts between low (10 ng) and high (100 ng) RNA inputs (Figures 3 and 4).

Notably, the AVITI System maintained high consistency in detecting long transcripts, including protein-coding genes and long noncoding RNAs, across replicates and different input levels. Additionally, the SEQuoia Complete Kit demonstrated proficiency in detecting small RNA transcripts (<200 bp), with consistent miRNA detection between input levels (Figure 5). Uniform read coverage across gene positions further underscores the lack of bias in sequencing, validating the quality and reliability of SEQuoia-prepared libraries on the AVITI platform (Figure 6). These findings highlight the AVITI System as a robust tool for comprehensive transcriptomic profiling across diverse RNA inputs and biotypes.

Sequence Quality

Triplicates of the SEQuoia Complete and SEQuoia Express–generated libraries were constructed for high and low RNA inputs and achieved highly reproducible sequencing on the Element AVITI System (Table 2). Overall, SEQuoia RNA-Seq library preparation on the AVITI System demonstrated excellent sequence quality, with both high and low RNA input.

Consistent Sequencing Results

In evaluations of the SEQuoia Express Stranded RNA Library Prep Kit, the Element AVITI System produced consistent sequencing results at either low (10 ng) or high RNA input (100 ng), as shown in Figure 3.

Consistent Long Transcript Detection Using the SEQuoia Complete Kit

A similar evaluation was also conducted using the SEQuoia Complete Stranded RNA Library Prep Kit on the AVITI System, as shown in Figure 4, and provided consistent sequencing results in long transcript detection among sample replicates and different input RNA.

High Correlation in miRNA Transcript Detection Across RNA Input Levels with the SEQuoia Complete Kit

The AVITI System run with the SEQuoia Complete Stranded Library Prep Kit shows robust performance in detecting miRNA transcripts, with high correlation ($R^2 = 0.986$) across low and high RNA inputs (Figure 5A). Moreover, the SEQuoia Complete Stranded Library Prep Kit enables sensitive detection of small RNA transcripts (<200 bp). The AVITI System also exhibits consistent miRNA detection across varying input amounts (10–100 ng), detecting similar miRNA profiles at ≥5 reads per transcript (Figure 5B), highlighting its comprehensive capability to capture both long and short RNA biotypes in a single sequencing approach.

Uniform Read Coverage

Exceptional read coverage uniformity was observed from the SEQuoia Complete Kit and SEQuoia Express Kit–generated libraries sequenced on the AVITI System (Figure 6). The read coverage uniformity is nearly identical for both the 5' and 3' ends across the entire transcript, demonstrating no bias in coverage and supporting the high quality of the SEQuoia Kit–generated RNA libraries sequenced on the AVITI platform.

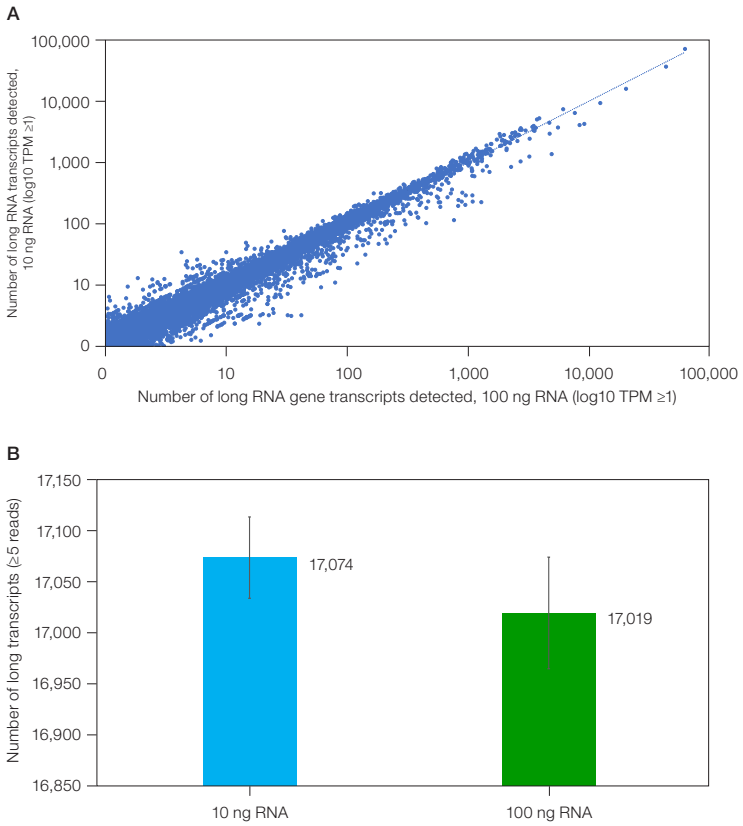


Fig. 3. SEQuoia Express libraries run on the AVITI System produce consistent sequencing results in long transcript detection among sample replicates and different input RNA, with a high correlation of transcripts detected from the triplicates of the SEQuoia Express library. A, a high correlation of long transcripts at TPM ≥1 with the SEQuoia Express Kit from RNA-Seq runs on the Element AVITI System. **B**, the number of long RNA transcripts obtained on AVITI System with high (100 ng) and low (10 ng) input RNA levels with the SEQuoia Express Kit at ≥5 reads per transcript and a 95% confidence interval. TPM, transcripts per million.

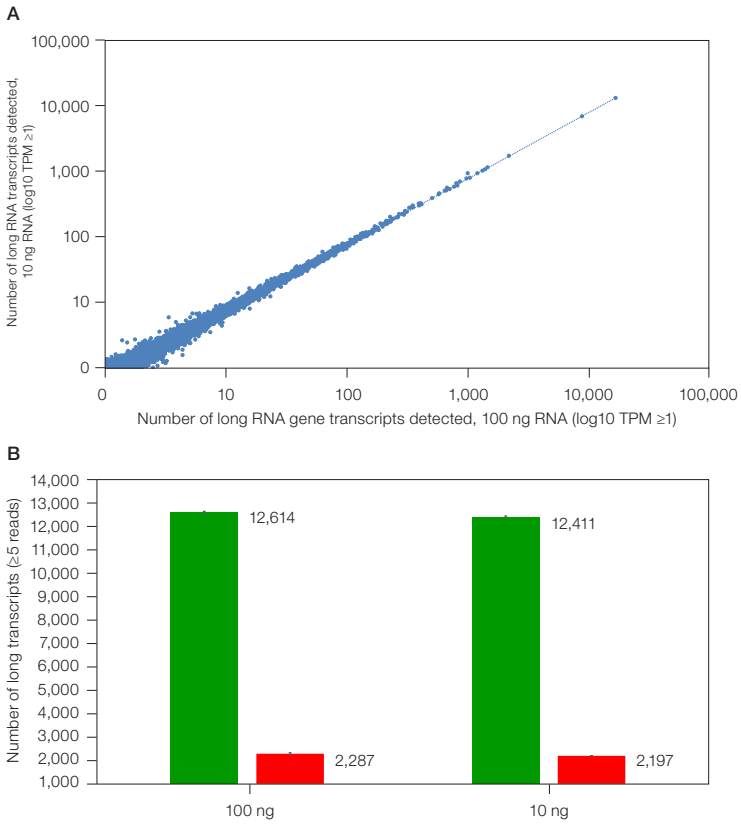


Fig. 4. SEQuoia Complete libraries run on the AVITI System produce consistent sequencing results in long transcript detection among sample replicates and different input RNA. A, a high correlation of long transcripts at TPM ≥1 with the SEQuoia Complete Kit from RNA-Seq runs using 10 ng and 100 ng input RNA on the Element AVITI System; **B**, the number of long RNA transcripts obtained on the AVITI System with high and low input RNA levels with the SEQuoia Complete Kit was comparable, at ≥5 reads per transcript and a 95% confidence interval. Long transcripts included mainly protein-coding and long noncoding genes. TPM, transcripts per million.

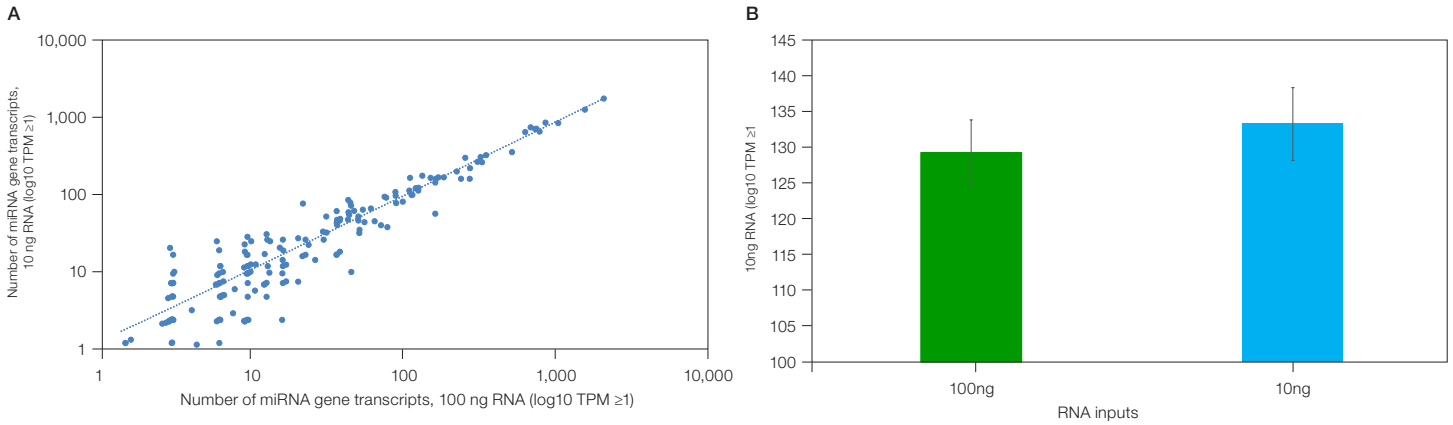


Fig. 5. Libraries generated with the SEQuoia Complete Kit run on the AVITI System produce consistent sequencing results in miRNA detection among sample replicates between different input RNA. The AVITI System run with the SEQuoia Complete Stranded Library Prep Kit shows robust performance in detecting miRNA transcripts, with excellent correlation ($R^2 = 0.986$) across low and high RNA inputs. Across varying input amounts (10–100 ng), the AVITI System detects similar miRNA profiles at ≥5 reads per transcript. **A**, a high correlation of miRNA transcripts at TPM ≥1 with the SEQuoia Complete Kit from RNA-Seq runs on the Element AVITI System. **B**, the number of miRNA transcripts obtained on the AVITI System, using high and low input RNA levels with the SEQuoia Complete Kit, was comparable at ≥5 reads per transcript and a 95% confidence interval. TPM, transcripts per million.

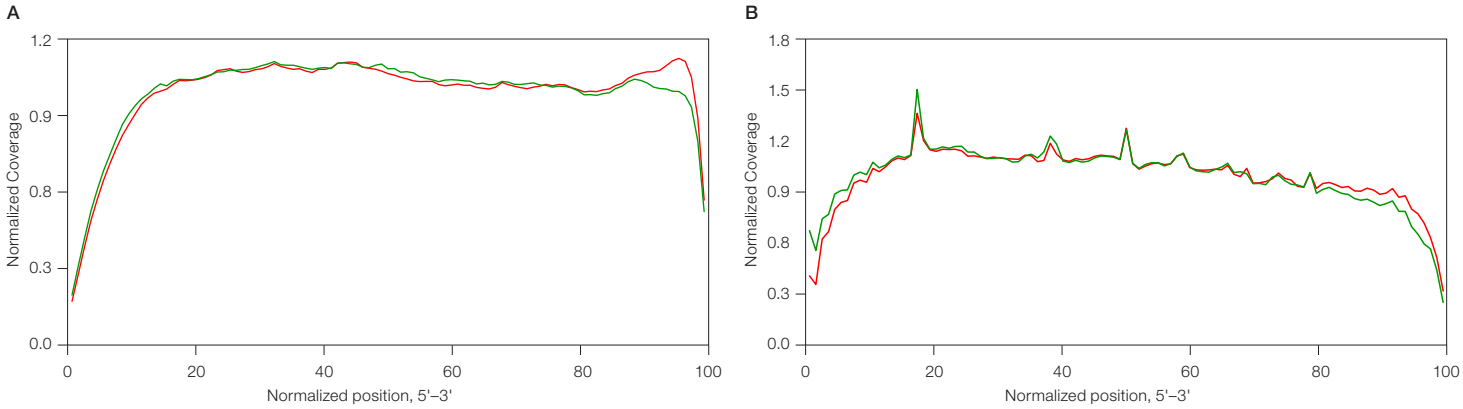


Fig. 6. Read coverage uniformity by position from the SEQuoia RNA-Seq libraries sequenced on the Element AVITI platform. Normalized coverage plots by gene position between the high and low RNA input for libraries prepared with **A**, the SEQuoia Express Stranded RNA Library Kit with 100 ng and 10 ng RNA input and **B**, the SEQuoia Complete RNA-Seq library with 100 ng and 10 ng RNA input.

Conclusions

The availability of non-Illumina sequencing platforms in the market reduces the overall cost of sequencing for researchers. Additional benefits include broader sequencing options for researchers, access to diversified technologies, and more tailored sequencing solutions, ultimately advancing scientific discovery and application.

This study establishes the compatibility of the SEQuoia bulk RNA-Seq library preparation kits with the Element AVITI platform, affirming the generation of high-quality sequencing data regardless of RNA input amounts, and with the use of sample sources containing varied RNA subtypes (demonstrated by UHR versus human placental total RNA). RNA-Seq libraries prepared using the SEQuoia Complete Stranded RNA Library Prep Kit and the SEQuoia Express Stranded RNA Library Kit consistently achieved high Q30 scores when sequenced on the AVITI System. Furthermore, an equivalent number of unique transcripts representing both long and miRNAs were identified in the SEQuoia Complete Stranded RNA Library Prep Kit sequenced on the platform between high and low RNA inputs, whereas the SEQuoia Express Stranded RNA Library Prep Kit enabled high data quality for the detection of long RNA transcripts, even with varying input. Notably, the exceptional read coverage uniformity across the 5' and 3' ends support the lack of bias and robust performance of the SEQuoia RNA libraries used for sequencing on the platform. The use of the Element AVITI System adapters in library construction obviates the need for separate dual index primers (DIPs), simplifying the workflow from library preparation to data analysis. Furthermore, this platform combination enables equivalent detection of both long transcripts and miRNAs across varying RNA inputs.

The broad application and robust performance of the Bio-Rad SEQuoia RNA library prep products together with the Element AVITI System offer a scalable and affordable option that can be easily tailored for more comprehensive transcriptome profiling. With options beyond a single sequencing technology provider, researchers can now optimize their experimental design by selecting the platform that offers the best performance for their specific RNA library preparation method (such as SEQuoia Kits) and biological samples, whether detecting long transcripts, miRNAs, or handling varying RNA input amounts. This flexibility enhances the quality and relevance of sequencing data generated for research questions, and adapts to changes in experimental conditions, sample types, or project requirements more effectively.

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Visit **bio-rad.com/SEQuoiaComplete** for more information on the SEQuoia Complete and SEQuoia Express Kits.

Visit **elementbiosciences.com/applications** for more information on the Element AVITI solutions for RNA sequencing

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Visit bio-rad.com/NGS for more information.

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