Element Biosciences

Application Note

MicroRNA Sequencing with the Element AVITI™ System

Avidity base chemistry delivers a high-quality, low-cost solution for profiling and discovery applications

Highlights

- High quality scores and high recovery
- Consistent correlation and expression of microRNA
- Scaling for small inserts and low output
- Compatible with third-party RNA library preps

Introduction

Next-generation sequencing (NGS) of microRNA (miRNA) gives researchers insight into key biological functions. These small, noncoding RNA molecules influence gene silencing and post-transcriptional regulation of gene expression, contributing to cellular development and function while also serving as biomarkers for disease.[1-3] Consistent, accurate data are critical for the many NGS applications that measure miRNA expression.[4]

The Element AVITI System delivers the required quality at a low cost per run. Avidity base chemistry (ABC) underpins AVITI sequencing, eliminating PCR error propagation, reducing reagent consumption, and readily scaling to fit the unique needs of miRNA.[5] Spanning a brief ~22 bp, miRNA is typically sequenced single-end and requires only ~5–10 million reads per sample. This application note demonstrates how AVITI advances a distinctive solution for miRNA sequencing.

Methods

Library preparation

The QIAseq miRNA Library Kit (QIAGEN) prepared libraries from inputs of 100 and 200 ng total RNA references. Two reference types were used, Human Brain Reference Total (HBR) RNA (Thermo Fisher Scientific) and Human XpressRef Universal Total RNA (XR) (QIAGEN), preparing six linear replicates per reference. The prep included unique dual indexes (UDIs) and unique molecular identifiers (UMIs). UMIs enable quantification of each miRNA molecule and mitigate the abundance bias that reverse transcription and amplification can introduce.[4]

Library adaption

The Element Adept™ Library Compatibility Workflow adapted the twelve replicates into AVITI-compatible libraries. To show concordance between different adaption methods, the linear replicates were split between two protocols:

- The Adept Rapid PCR-Free protocol circularized 12 replicates, six of each input type, generating **circular libraries**.
- The Adept Rapid PCR-Plus protocol amplified 12 replicates, six of each input type, generating **linear libraries** for automatic circularization onboard AVITI.

Sequencing

After adaption, the circular and linear libraries were separately pooled. The circular library pool was diluted to 5 pM and loaded onto AVITI with a spike-in of PhiX Control Library, Adept at 0.1 pM.

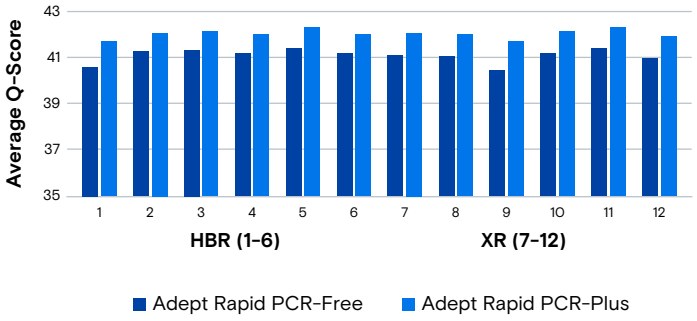

Figure 1. The high average Q-score of UMI reads prepared from HBR and XR and sequenced on AVITI.

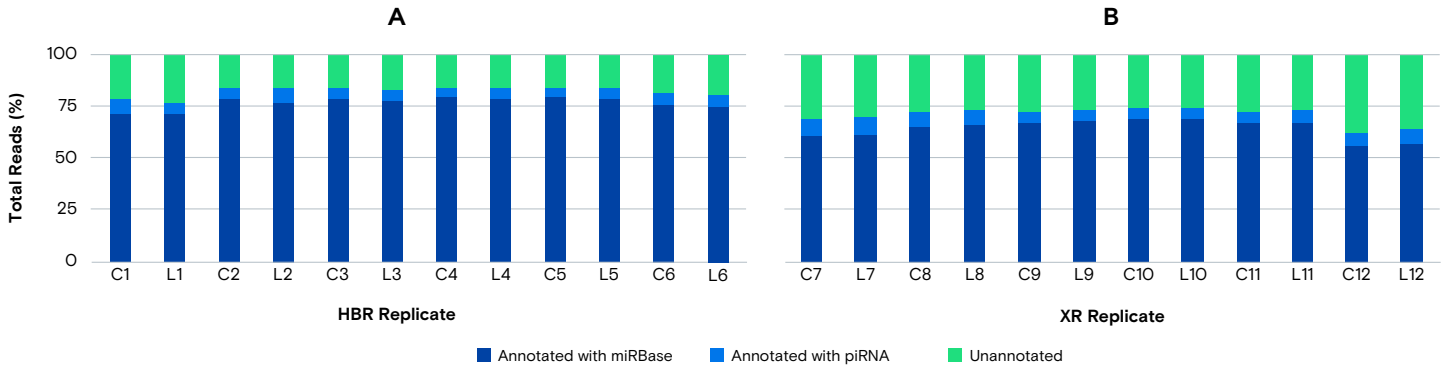

Figure 2. Mapping metrics for HBR (A) and XR (B) show a high percentage of total reads mapped for both adaption methods. Replicates C1–C12 denote the Adept Rapid PCR-Free protocol and replicates L1–L12 denote Adept Rapid PCR-Plus.

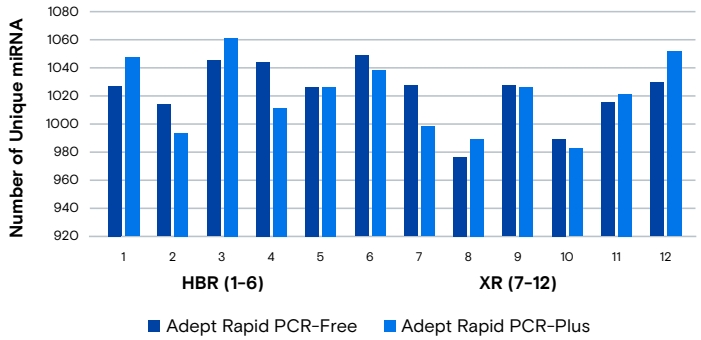

Figure 3. miRBase annotated a high number of unique mRNA overall and each adaption method presented similar results.

Using the same spike-in, the linear library pool was diluted to 6 pM for loading and sequencing in a separate run. Both runs used the same read length and sequencing kit: 1 x 72 with two 10 bp index reads and the 2 x 150 Sequencing Kit Cloudbreak™ High Output.

Data analysis

Bases2Fastq Software demultiplexed the AVITI-generated bases files and output FASTQ files. To ensure comparison at a read depth common to miRNA studies, the data were downsampled to ~10 million reads per sample. The FASTQ files were uploaded to QIAGEN RNA-seq Analysis Portal 4.1 for miRNA analysis. The portal merged the UMI reads, then counted and annotated miRNA molecules using miRBase. Expression levels were generated for mature miRNA.

All analyses used the default portal settings for miRNA samples and followed the QIAGEN-supplied protocol. The default settings included annotation with miRBase and the normalization of differential expression and counts per million (CPM) data via trimmed mean of M-values (TMM). Genome assembly GRCh38 served as the alignment reference for all samples.

Results

Overall sequencing quality

AVITI sequenced all replicates at high quality, delivering an average quality score (Q-score) of 41–42 (Figure 1). Additionally, each run achieved a total read count of 700–900 million. Each sample reached ~60 million reads. Although output > 10 million reads per sample is not necessary for miRNA, a high read count demonstrates capability to achieve both high quality and high output. Rightsize your miRNA sequencing with a 2 x 75 kit.

Recovery of miRNA molecules

Mapping reveals how much miRNA is recovered from total RNA input. AVITI mapping is high, demonstrating both compatibility and accuracy. This result confirms that miRNA represents the majority of reads and is consistent across sample replicates regardless of adaption method. Results remained high overall, varying somewhat by reference type due to the distinct composition of each.

An average ~71% of total reads were mapped across all samples:

- In HBR, miRNA represented ~77% of sequencing and UMI reads (Figure 2A).
- In XR, miRNA represented ~65% of sequencing and UMI reads (Figure 2B).

Two other measures of miRNA recovery, annotation and abundance, demonstrate similarly strong results as mapping with elevated counts of unique miRNA molecules and high abundance. Annotation queries miRBase to identify and count the mature miRNA found in each sample. For HBR, miRBase annotated an average of 1031 unique miRNA molecules. The XR average was 1011. Figure 3 shows the total number of unique miRNA molecules annotated for each sample.

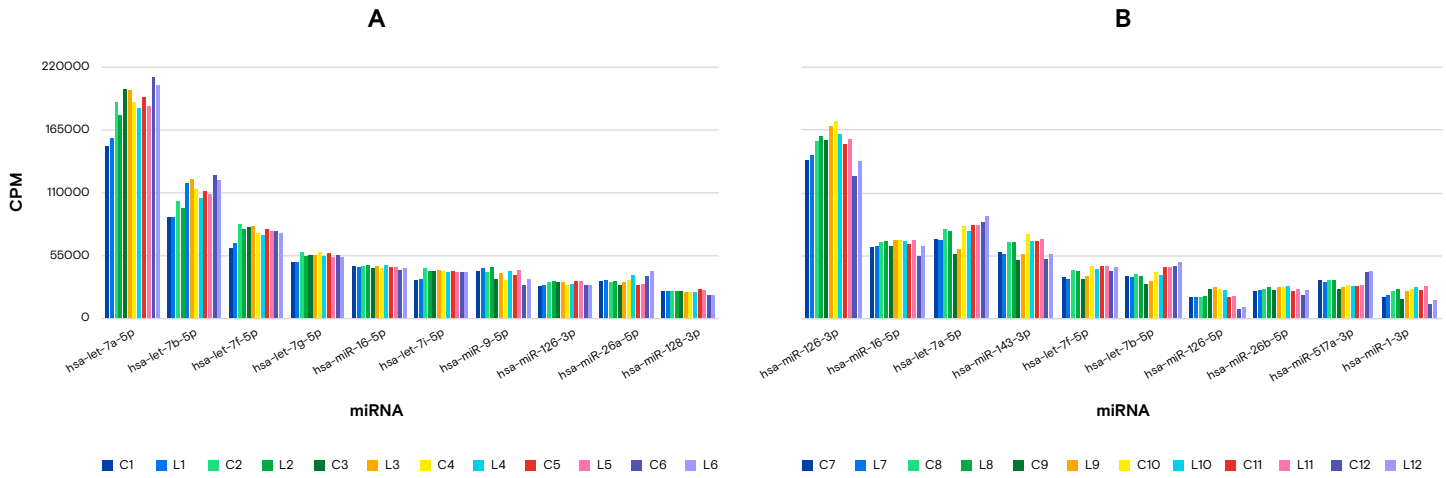

Figure 4. The 10 most abundant miRNA from HBR (A) and XR (B). Replicates C1–C12 denote the Adept Rapid PCR-Free protocol and replicates L1–L12 denote Adept Rapid PCR-Plus.

Abundance—or counts per million (CPM)—across replicates was largely consistent, showing only minor variation. As expected given the different content of each reference, the most abundant miRNA molecules varied between HBR and XR. Figure 4 shows CPM for the 10 most abundant molecules in each reference.

Expression values correlation

Expression values assess and compare miRNA activity among samples or between groups of samples. The expression values described in this study indicate extremely high correlation between the two sequencing runs: R^2 = 0.9973 (Figure 5). Results for each input reference (HBR and XR) and replicates were similar. The lack of appreciable expression difference between manually and automatically circularized libraries suggests that both adaption methods are suitable choices to prepare miRNA libraries for ABC sequencing.

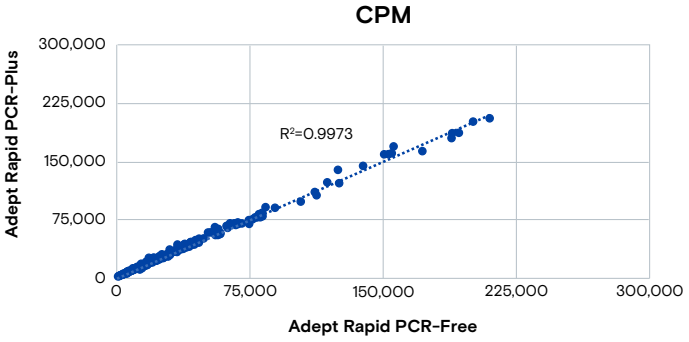

Figure 5. TMM-normalized expression values demonstrate high correlation between PCR-plus linear libraries PCR-free circular libraries.

Conclusion

NGS is a wide-ranging and invaluable tool for a spectrum of miRNA studies, enabling miRNA quantification, identification of novel miRNA, detection of differences in miRNA sequences, and detection of miRNA from many samples simultaneously.[6] AVITI advances these gains by upholding quality expectations while reducing the price point to deliver a solution flexible enough to explore both existing and novel miRNA. AVITI is robust against variable input and library prep methods, with high-correlation results demonstrating that ABC sequences manually and automatically circularized miRNA libraries equally well.

To learn more, visit elementbiosciences.com

For us, sequencing is only the beginning.

At Element Biosciences, our mission is to empower the scientific community with more freedom and flexibility to accelerate our collective impact on humanity. We're focused on innovating genetic analysis tools for the research market. Our proprietary approach to improving the signal-to-noise ratio allows us to provide groundbreaking innovations in surface chemistry, instrumentation, and biochemistry to drastically decrease the run cost and capital cost while delivering high sequencing data quality.

References

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