illumina®

TECHNICAL NOTE

miRNA expression profiling with Illumina miRNA Prep

Enrichment, reproducibility, and sequencing performance for miRNA libraries

Enrich for miRNA across diverse input types

Most sequencing reads are assigned to miRNA, as determined by UMI-enabled DRAGEN™ secondary analysis

Generate consistent miRNA libraries

Libraries demonstrate reproducible concentrations and fragment sizes across input types

Achieve reliable sequencing

The MiSeq™ i100 Plus and NextSeq™ 2000 Systems deliver consistent quality metrics

Introduction

MicroRNAs (miRNAs) are small, noncoding RNAs that regulate gene expression at the posttranscriptional level. By modulating the stability or translation of target mRNAs, miRNAs influence a wide range of biological processes and can act within cells or circulate in biofluids. Through coordinated regulation of multiple gene targets, miRNAs play important roles in development, cellular homeostasis, and disease biology.[1]

Changes in miRNA expression have been associated with many human diseases, including cancer and rare genetic disorders.[2,3] As a result, miRNAs are widely studied both as regulators of gene expression and as molecular biomarkers that reflect disease state, tissue origin, or therapeutic response. Reliable measurement of miRNA expression is therefore central to applications such as biomarker discovery, gene regulation research, and drug discovery and development.

Next-generation sequencing (NGS) enables comprehensive profiling of miRNA expression with high specificity and dynamic range, supporting detection of low-abundance miRNAs and discrimination among closely related sequences.[4] At the same time, the short length of miRNAs and limited RNA input amount typical of many sample types can introduce technical bias during library preparation, affecting quantification accuracy and reproducibility.

Illumina miRNA Prep is designed to support scalable miRNA sequencing across a broad range of research applications and RNA inputs. The workflow enables focused interrogation of the miRNA fraction from total RNA without gel-based size selection, helping to streamline library preparation and reduce sample loss. Integration of unique molecular identifiers (UMIs) supports more accurate miRNA counting by distinguishing original miRNA molecules from other types of RNA and amplification artifacts.[5]

This technical note demonstrates the performance of Illumina miRNA Prep for miRNA sequencing using RNA derived from multiple sample sources and sequenced on the MiSeq™ i100 Plus and NextSeq™ 2000 Sequencing Systems.

Methods

Samples

Samples from multiple sources, including whole blood, and tissue, were sourced commercially (Table 1). For whole blood, total RNA was extracted using the QIAsymphony PAXgene Blood RNA Kit (QIAGEN, Catalog no. 762635) according to manufacturer instructions.

Table 1: RNA samples and input sources

Sample	Description	Vendor	Catalog no.
Human brain total RNA	Human brain total RNA	Thermo Fisher Scientific	AM7962
Human liver total RNA	Human liver total RNA	Thermo Fisher Scientific	AM7960
Human blood (donor 1)	Human whole blood (gender unspecified)	BioIVT	HUMANWBPAXR-0104957
Human blood (donor 2)	Human whole blood (gender unspecified)	BioIVT	HUMANWBPAXR-0104957

Library preparation and sequencing

Sequencing libraries were prepared manually according to the Instructions for using the Illumina miRNA Prep kit, using 100 ng total RNA input for each sample. The hands-on time for Illumina miRNA Prep from adapter ligation through library clean-up is approximately 4–5 hr, as summarized in the workflow overview (Figure 1).

Libraries were prepared without gel-based size selection and incorporated UMIs during reverse transcription.

Sequencing was performed on the MiSeq i100 Plus and NextSeq 2000 Systems using a 72 × 10 × 10 run configuration (Read 1 × Index 1 × Index 2).

Illumina recommends sequencing miRNA libraries at a depth of 5–10 M reads per sample. Additional guidance on sample loading and number of samples per flow cell is available at Project recommendations for Illumina miRNA Prep.

Data analysis

Primary and secondary analysis of miRNA sequencing data was performed using DRAGEN miRNA on BaseSpace™ Sequence Hub.

Results

Library quality and yield

Library preparation quality was first assessed across all sample types using standard quality control (QC) metrics. Libraries prepared with Illumina miRNA Prep showed consistent concentration and fragment size, and molarity across two replicate preparations for each sample (Table 1 and Figure 2). All libraries achieved concentrations of ≥ 30 nM, with consistent values observed across sample types and replicates, supporting uniform library generation from diverse RNA inputs.

Primary sequencing metrics

Sequencing performance metrics were evaluated for pooled libraries sequenced on both the MiSeq i100 Plus and NextSeq 2000 Systems (Table 3). Each sequencing pool consisted of two replicate library preparations for each of the six sample types. Key run-level metrics, including total reads, percent passing filter (%PF, percentage of clusters passing quality filters), and percent ≥ Q30 (%Q30, percentage of bases with a quality score ≥ 30), met expected performance specifications for miRNA sequencing on both systems.

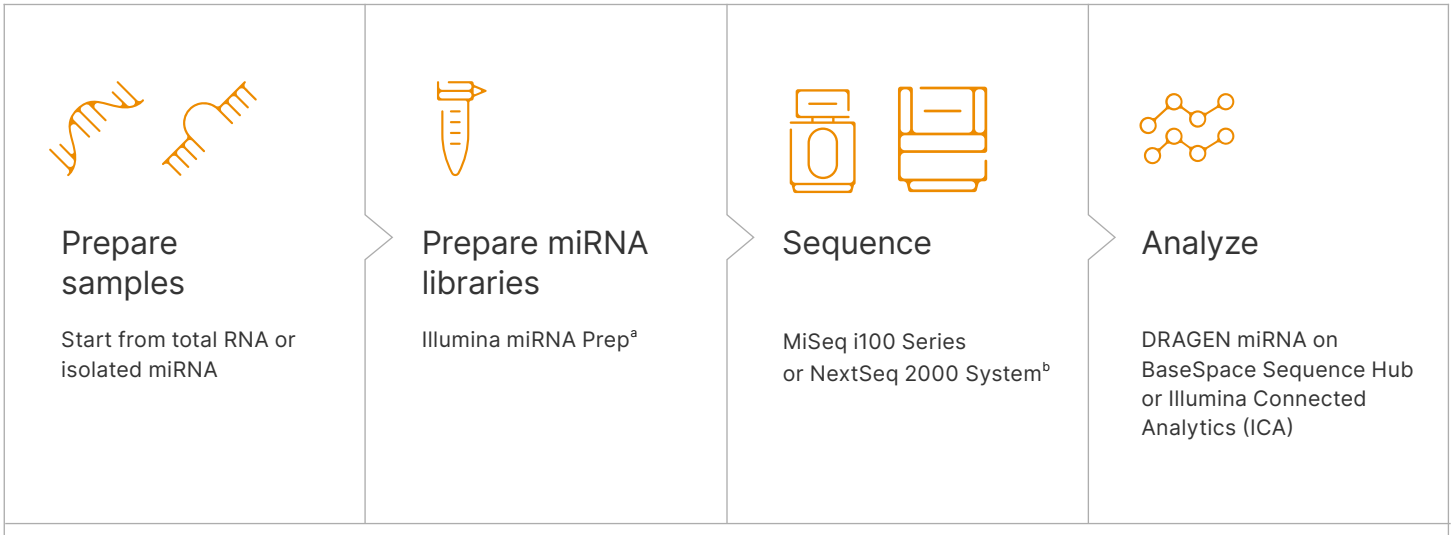

Figure 1: Illumina miRNA Prep workflow
Total RNA or isolated miRNA is used to generate miRNA-specific libraries. Illumina BaseSpace Sequence Hub provides software tools for primary and secondary analysis.

a. Hands-on time from adapter ligation to library clean-up is ~4–5 hr.
b. Using 384 unique dual indexes. Between 5–10M reads/sample are recommended based on input sample type and experimental design.

Table 2: QC results for libraries prepared with Illumina miRNA Prep

Sample	Replicate	Concentration (ng/µl)	Average fragment size[a] (bp)
Human brain total RNA	1	13.3	223.5
	2	14.9	226.0
Human liver total RNA	1	7.8	223.5
	2	5.3	198.0
Human blood (donor 1)	1	4.5	219.0
	2	4.1	218.5
Human blood (donor 2)	1	4.5	210.0
	2	4.1	213.0

a. 25–1000 bp window.

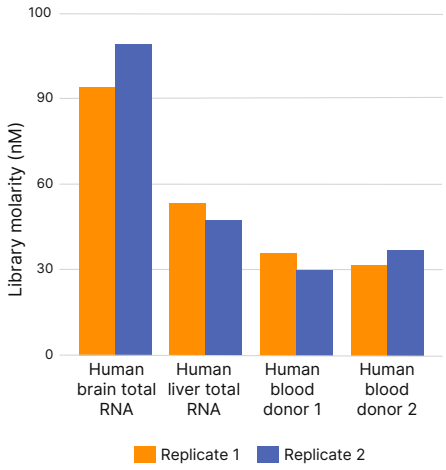

Figure 2: Concentrations of sequencing libraries prepared with Illumina miRNA Prep

Library molarity (nM) is shown for sequencing libraries prepared from two replicates of total RNA extracted from human brain, human liver, human whole blood (donor 1), and human whole blood (donor 2) samples (Table 1).

Table 3: Sequencing metrics for pooled libraries prepared with Illumina miRNA Prep

Library pool	Sequencing system (flow cell)	%PF[a]	%QC[b]
A	MiSeq i100 Plus (100M)	74.2	96.7
	NextSeq 2000 (P3)	89.1	96.6
B	MiSeq i100 Plus (100M)	69.5	97.2
	NextSeq 2000 (P3)	76.4	96.1

a. Percentage of clusters passing quality filters.
b. Percentage of bases with a quality score ≥ 30.

miRNA distribution

Secondary analysis using DRAGEN miRNA on BaseSpace Sequencing Hub was used to determine the RNA biotype composition for pooled miRNA libraries on the Illumina MiSeq i100 Plus and NextSeq 2000 Systems (Figure 3 and Figure 4, respectively). miRNA reads represented the predominant RNA biotype across pooled libraries for most sample types. Mapping rates exceeded 50% for human brain total RNA, human liver total RNA, and human whole blood samples, consistent with expectations for bulk RNA inputs.

The DRAGEN miRNA app applies data QC steps to remove reads that lack adapters, are shorter than 16 bp, or contain UMIs shorter than 10 bp. For human, mouse, and rat samples, the remaining reads are mapped to species-specific miRBase and piRNABank reference databases, followed by alignment to the reference genome to identify potential novel miRNAs. Candidate novel miRNAs can be found in the "notCharacterized_mappable" output file, which contains reads that do not map to the reference databases but do map to the genome.

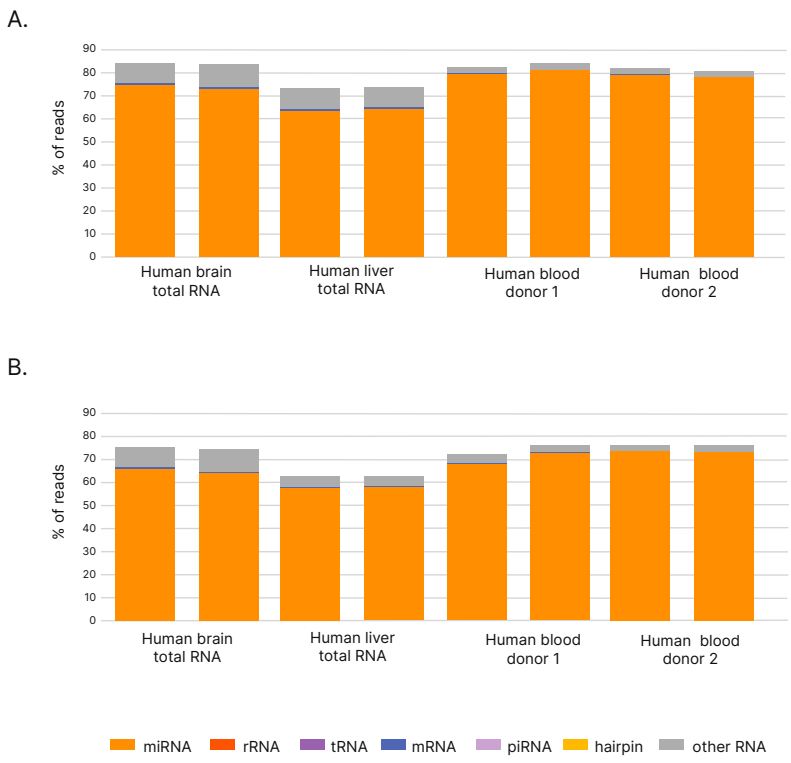

Figure 3: RNA biotype distribution for pooled miRNA libraries sequenced on the MiSeq i100 Plus System

RNA biotype composition was assessed using DRAGEN secondary analysis for (A) library pool A and (B) library pool B (Table 3) sequenced on the MiSeq i100 Plus System (100M flow cell).* The percentage of reads assigned to miRNA, rRNA, mRNA, piRNA, hairpin reads, and other RNA species is shown for each sample type, with UMIs used to distinguish individual samples within the pool.

mRNA, messenger RNA; miRNA, microRNA; rRNA, ribosomal RNA; tRNA, transfer RNA;

* Illumina recommends up to 24 miRNA libraries per 100M flow cell at 4M average reads/sample.

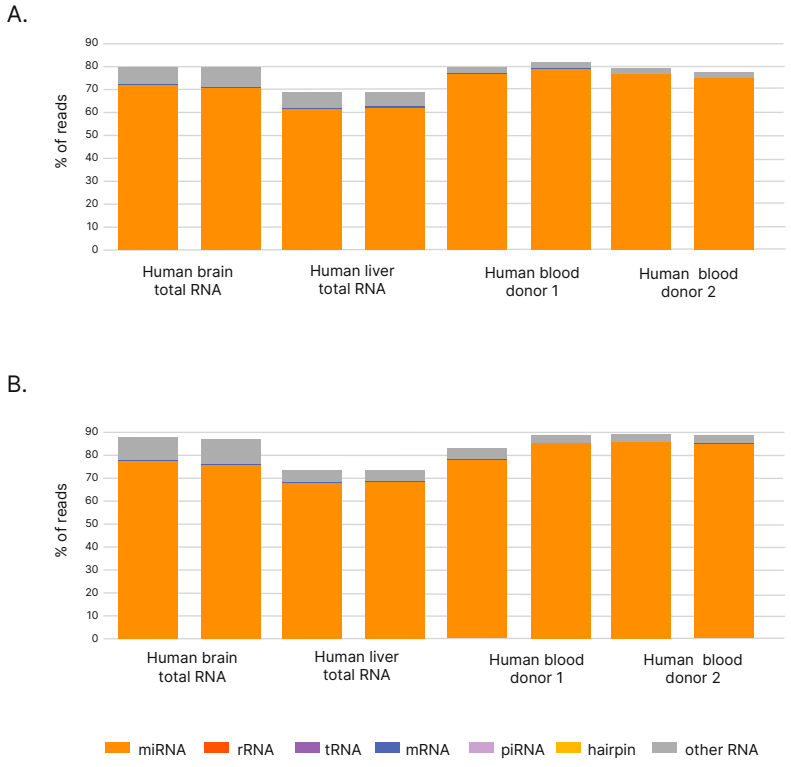

Figure 4: RNA biotype distribution for pooled miRNA libraries sequenced on the NextSeq 2000 System

RNA biotype composition was assessed using DRAGEN secondary analysis for (A) library pool A and (B) library pool B (Table 3) sequenced on the NextSeq 2000 System (P3 flow cell). The percentage of reads assigned to miRNA, rRNA, mRNA, piRNA, hairpin reads, and other RNA species is shown for each sample type, with UMIs used to distinguish individual samples within the pool.

mRNA, messenger RNA; miRNA, microRNA; rRNA, ribosomal RNA; tRNA, transfer RNA; piRNA, PIWI-interacting RNA.

miRNA expression concordance between sequencing systems

To evaluate consistency of miRNA expression measurements across sequencing systems, pooled miRNA libraries generated from duplicate samples were sequenced on both MiSeq i100 Plus and NextSeq 2000 Systems. miRNA expression levels showed a strong linear correlation between systems (R^2 = 0.996), indicating consistent miRNA expression profiling across sequencing systems (Figure 5).

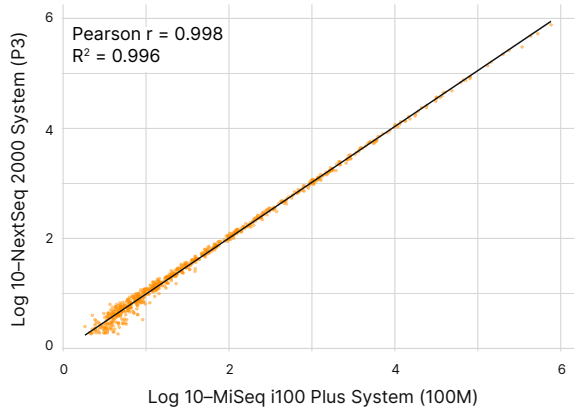

Figure 5: Correlation of miRNA expression between sequencing systems
Correlation of miRNA expression measured on the MiSeq i100 Plus System (100M flow cell) and the NextSeq 2000 System (P3 flow cell) using pooled miRNA libraries generated from duplicate samples.

Summary

Illumina miRNA Prep generated consistent miRNA sequencing libraries across multiple RNA sample types, with reproducible library concentrations and fragment sizes observed across replicate preparations. Runs on the MiSeq i100 Plus and NextSeq 2000 Systems met expected run performance metrics. Secondary analysis showed miRNA as the predominant RNA biotype across pooled libraries, with consistent biotype distributions across samples. miRNA expression measurements demonstrated reproducible miRNA expression profiling across Illumina sequencing systems.

Learn more →

Illumina miRNA Prep

DRAGEN miRNA on BaseSpace Sequence Hub

References

1. O'Brien J, Hayder H, Zayed Y, Peng C. Overview of microRNA biogenesis, mechanisms of actions, and circulation. *Front Endocrinol*. 2018;9. doi:10.3389/fendo.2018.00402
2. Sundarbose K, Kartha RV, Subramanian S. MicroRNAs as biomarkers in cancer. *Diagnostics*. 2013;3(1):84-104. doi:10.3390/diagnostics3010084
3. Goel H, Goel A. MicroRNA and rare human diseases. *Genes*. 2024;15(10). doi:10.3390/genes15101243
4. Liu J, Jennings SF, Tong W, Hong H. Next generation sequencing for profiling expression of miRNAs: technical progress and applications in drug development. *J Biomed Sci Eng*. 2011;4(10):666-676. doi:10.4236/jbise.2011.410083
5. Illumina. Illumina miRNA Prep data sheet. illumina.com/content/dam/illumina/gcs/assembled-assets/marketing-literature/illumina-mirna-prep-datasheet-m-gl-03399/illumina-mirna-prep-datasheet-m-gl-03399.pdf. Published 2025. Accessed February 16, 2026.

1.800.809.4566 toll-free (US) | +1.858.202.4566 tel
techsupport@illumina.com | www.illumina.com

M-GL-04160 v1.0