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Complete Small RNA Sequencing Solutions on the MiniSeq™ System

Uncover the complete picture of small RNA expression regulation with the power of next-generation sequencing in a simple, personal platform.

Highlights

- **Efficient, Multiplexed Small RNA Sequencing**
  Accelerate your research by studying thousands of small RNA sequences simultaneously with greater sensitivity and dynamic range

- **Accessible, Affordable NGS Technology**
  Access a personal, reliable sequencing platform for low-throughput RNA sequencing solutions

- **Simple, Streamlined End-to-End Solution**
  Integrated workflows from library preparation to easy push-button data analysis options

- **Industry-Leading Data Quality**
  Build confidence in your results with the most widely adopted, and proven Illumina sequencing chemistry[1]

Figure 1: Small RNA Sequencing—Study thousands of miRNA and small RNA sequences on the MiniSeq System with greater sensitivity and dynamic range.

Introduction

Small RNAs, including microRNAs (miRNAs), are single stranded noncoding RNAs between 17-34 nucleotides in length and have been implicated as key gene regulators in many cellular pathways (Figure 1). Small RNAs are involved in the regulation of numerous cellular processes including circadian rhythms and viral regulation.[1,2] The MiniSeq System offers the easiest and most affordable entry into the realm of next-generation sequencing (NGS) for small RNA sequencing (RNA-Seq) applications.

NGS makes sequence-based expression analysis a "digital" alternative to analog techniques. Microarray gene expression measurements are limited by noise and signal saturation. In contrast, next-generation sequencing quantifies discrete, digital read counts, offering a large
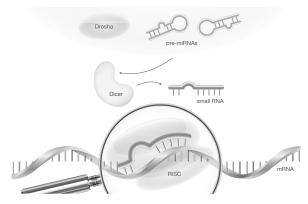
dynamic range. This enables highly scalable sensitivity, allowing researchers to tune the level of resolution to meet specific experimental needs. With the MiniSeq Small RNA-Seq workflow, millions of small RNA and microRNA (miRNA) sequences can be queried with unprecedented sensitivity and dynamic range. In addition to expression and profiling applications, the MiniSeq System is a powerful tool for the discovery of novel miRNAs and other small noncoding RNAs. Even without a reference sequence or secondary structure information, small RNA-Seq allows the characterization of variations such as isomiRs (miRNA variants) at single-base resolution.

Simple, Integrated Workflow

The MiniSeq System Small RNA-Seq workflow delivers a complete sample-to-answer workflow for the analysis of miRNAs and other noncoding RNAs (Figure 2).
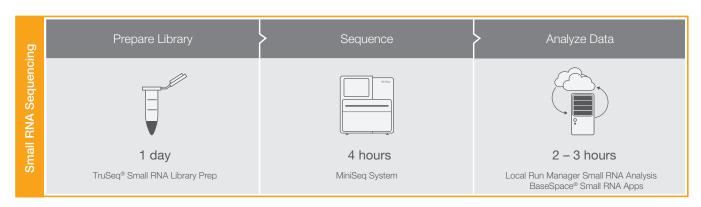

Figure 2: MiniSeq Small RNA-Seq Workflow—The integrated workflow enables streamlined library preparation for cost-effective studies covering all small RNA transcripts in any species.

Advantages of RNA-Seq Over Microarrays

- Requires no prior knowledge of the transcriptome

- Provides *qualitative* and *quantitative* data

- Enables deep sequencing for greater sensitivity and virtually unlimited dynamic range

- Delivers sequence and variant information

- Provides greater accuracy of fold-change measurements

- A better value than array technologies, delivering more data at a lower price per sample

Library Preparation

The small RNA-Seq workflow begins with TruSeq Small RNA Library Prep Kit, which provides all reagents required to prepare the RNA libraries for sequencing, including up to 48 individual indexes for multiplexing. The TruSeq Small RNA Library Preparation Kits provide a simple, cost- effective method for generating small RNA libraries directly from total RNA (Figure 3). Small RNAs generated by Dicer processing are efficiently targeted by modified adapters. The kits also support customizable size selection to investigate any small RNA between 17-35 nucleotides. These kits also enable multiplexing with up to 48 unique indexes, allowing efficient study design and maximum throughput for microRNA and small RNA sequencing runs. Indexes are added in a universal amplification reaction, greatly reducing ligation bias and ensuring accurate measurement of small RNA expression.

Sequencing on the MiniSeq System

The MiniSeq System is designed for easy, streamlined operation (Figure 4). For quick, load-and-go set up, the MiniSeq Reagent Kits provide a flow cell, wash reagents, and a single reagent cartridge preloaded with all required sequencing reagents. The MiniSeq Reagent Kits are available in Mid-Output and High-Output formats, allowing optimization of study designs based on read-length, sample number, and output requirements.

The MiniSeq System offers an intuitive touch screen interface that provides simple, step-by-step guidance through each stage of the sequencing run, including library and reagent loading, run configuration, and run monitoring. MiniSeq Control Software performs onboard image analysis, base calling, and quality scoring. Quality statistics from 1 or multiple runs can be monitored in real time using the Sequencing Analysis Viewer (SAV) software. SAV software can be used onboard the sequencing instrument, or can be accessed from any location with a Windows-based PC.

Simplified Bioinformatics

Data analysis with the MiniSeq System requires no informatics expertise or command-line experience. The MiniSeq System features Local Run Manager software, an on-instrument system for creating a run, monitoring status, and analyzing sequencing data. With Local Run Manager, on-instrument data analysis can be automatically performed upon completion of the sequencing run. The data analysis modules generate simple reports for a wide range of sequencing applications.

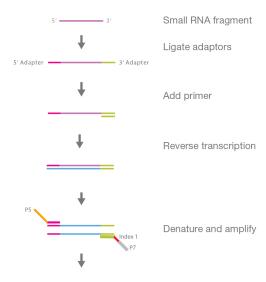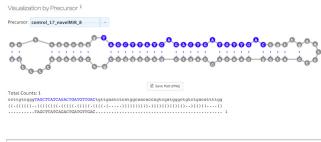## TruSeq Small RNA Chemistry

Figure 3: TruSeq Small RNA-Seq Library Preparation Chemistry—The TruSeq Small RNA Library Preparation Kits provide a simple, cost- effective method for generating small RNA libraries directly from total RNA.

Figure 4: MiniSeq System—The MiniSeq System leverages the latest advances in Illumina sequencing chemistry and an easy, integrated workflow.

Table 1: Small RNA-Seq Sample Throughput on the MiniSeq System

Study Measurement	Reads Per Sample	Samples Per Run	
		8 M	25 M
Differential expression of all small RNAs in a sample	2 M (1 × 50 bp)	4	12

Figure 5: Small RNA-Seq Analysis in BaseSpace—The Small RNA v1.0 App in BaseSpace enables visualization of small RNA precursors, mature miRNAs, and isomiR hits.

Figure 6: Small RNA Length Distribution—Review small RNA length distribution with the Small RNA v1.0 App in BaseSpace

The modular design allows users to install and update individual analysis modules as needed. In addition, sequencing data generated with the MiniSeq System can be instantly transferred, stored, and analyzed in the BaseSpace Computing Environment (cloud-based or onsite). BaseSpace RNA-Seq software Apps provide expert-preferred data analysis tools packaged in an intuitive, click-and-go user interface designed for informatics novices. The Small RNA BaseSpace App[3] aligns reads and performs optional novel precursor discovery as well as pairwise differential expression analysis (Figure 5 and Figure 6). Pairwise differential expression analysis identifies differentially expressed miRNAs, precursor groups, miRNA families, and piRNAs for each pair of sample groups.

For downstream analysis, BaseSpace Apps generate output files that can be directly input into a broad range of data analysis tools. The BaseSpace Environment includes a growing community of developers who use and provide software tools for visualization, analysis, and sharing. This NGS ecosystem provides one of the largest collections of commercial and open-source analysis tools currently available.

Small RNA-Seq vs Microarrays

Compared to microarrays, RT-PCR, and other traditional technologies, NGS small RNA-Seq offers several advantages. Small RNA-Seq allows deep sequencing, which provides higher sensitivity and a virtually unlimited dynamic range. This sensitivity enables superior performance and greater accuracy of fold-change measurements. Furthermore, sequencing provides in-depth, base-by-base coverage over the area of interest, which offers a more comprehensive approach for discovery applications or for the measurement of rare transcripts compared to traditional methods.
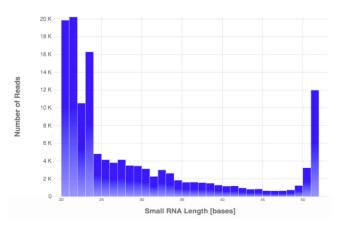
Summary

The MiniSeq System Small RNA-Seq Solution combines innovative engineering with proven sequencing chemistry to deliver a clear and complete view of miRNAs and other small RNAs. With the smallest footprint of any Illumina sequencing platform, the MiniSeq makes NGS technology more accessible to more researches and laboratories than ever before. By harnessing the power of NGS for small RNA sequencing, researchers can improve the accuracy and sensitivity of their data while simultaneously reducing costs. Accelerate your research and obtain your goals sooner with the MiniSeq Small RNA Sequencing Solution.

Learn More

To learn more about small RNA-Seq, go to www.illumina.com/techniques/sequencing/rna-sequencing/small-rna-seq.html.

For more on the TruSeq Small RNA Library Prep Kit, go to www.illumina.com/products/truseq_small_rna_sample_prep_kit.html.

Ordering Information

Sequencing System	Catalog No.
MiniSeq System	SY-420-1001
Sequencing Kits	
MiniSeq High Output Kit (75 Cycles)	FC-420-1001
MiniSeq Mid Output Kit (300 Cycles)	FC-420-1004
Small RNA Library Prep Kits[a]	
TruSeq Small RNA Library Prep Kit – Set A (24 samples)	RS-200-0012
TruSeq Small RNA Library Prep Kit – Set B (24 samples)	RS-200-0024
TruSeq Small RNA Library Prep Kit – Set C (24 samples)	RS-200-0036
TruSeq Small RNA Library Prep Kit – Set D (24 samples)	RS-200-0048

a. 24 sample kits contain 12 indexes

References

1. Flintoft L. MicroRNAs needed for time delay. *Nature Reviews Genetics.* 2013;14:746 (www.nature.com/nrg/journal/v14/n11/full/nrg3611.html). Accessed15 December 2015.
2. Lusic M, Marini B, Ali H, Lucic B, Luzzati R, Giacca M. Proximity to PML nuclear bodies regulates HIV-1 latency in CD4+ T cells. *Cell Host Microbe*. 2013;13:665-7.
3. Small RNA BaseSpace App (www.illumina.com/informatics/research/sequencing-data-analysis-management/basespace/basespace-apps/small-rna-948948.html). Accessed on 15 December 2015.

Maximize Performance and Productivity with Illumina Services, Training, and Consulting

Whether immediate help is needed during an instrument run, or in-depth consultations are required for sophisticated workflows, Illumina can help. Illumina service and support teams provide a full suite of expedient, customized solutions from initial trainings, to instrument support, and ongoing NGS education. Our support offerings include:

Illumina Professional Care Services Packs

Illumina offers Professional Care Services Packs - allotments of points that can be redeemed for discounted Illumina Professional Services. Benefits include:

- One-time Investment - no need for additional, postsale expenditures
- Risk Mitigation - bank points for unanticipated future services
- Savings - cost-effective versus a la carte pricing

Professional Care Services

Product Care Services

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- Instrument Compliance Services
- Instrument On-Demand Services

Illumina University Training

- Instructor-Led Training at Your Chosen Facility
- Instructor-Led Training at an Illumina Training Center
- Online Courses and Webinars

Illumina Consulting

- Proof-of-Concept Services for instrument and library preparation testing
- Concierge Services for design assistance and product optimization

For more on Illumina support offerings, visit: www.illumina.com/services/instrument-services-training.html

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