

MiSeq Focus: TruSeq® Targeted RNA Expression Solution for the MiSeq® System

Flexible, simple, and fully optimized gene expression assay for the MiSeq System.

-Highlights

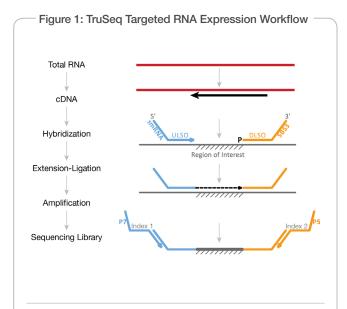
- Focused and Customizable Content for Maximum Flexibility
 - Combine fixed panels focused on biological pathways or disease-specific markers with custom content
- Fully Integrated RNA-to-Data Solution
 Streamlined, fully integrated workflow from library preparation to final data analysis
- Highly Multiplexed for Rapid, Efficient Results
 Supports 384 samples per run and 12–1000 assays per sample

Introduction

TruSeq Targeted RNA Expression on the MiSeq System delivers a simple and accurate method for measuring gene expression levels across thousands of targets in a single sequencing run. Library preparation begins with standard RNA isolation and reverse transcription reactions and is completed with one of several TruSeq Targeted RNA Expression Panel Kits (Figure 1). Dual indexing during library preparation enables highly multiplexed gene expression profiling for 12–1,000 assays* per sample and up to 384 samples per MiSeq System run.

With the highly multiplexed TruSeq Targeted RNA Expression Solution, the amount of processing time and hands-on time is significantly reduced. In a single run, the MiSeq System can generate 25 million reads or 25,000 datapoints (for example, with 50 multiplexed samples and 500 assays per sample) in less than two days (Figure 2). An equivalent number of data points using traditional qPCR methods would require 65 runs and ~16 days (using 384-well plates). Additionally, all targets are amplified in a single reaction, minimizing potential amplification bias compared to traditional methods.

TruSeq Targeted RNA Expression on the MiSeq System leverages Illumina sequencing by synthesis (SBS) technology, the most highly adopted and trusted next-generation sequencing technology in the industry.²⁻⁴ With an intuitive touch-screen interface and step-by-step guided workflows, Targeted RNA Expression panels offer a quick and easy solution for RNA expression profiling or gene expression array validation.



The TruSeq Targeted RNA Expression assay chemistry begins with reverse transcribing cDNA from purified total RNA. Two custom-designed oligonucleotide probes with adapter sequences hybridize up and downstream of the region of interest. An extension-ligation reaction, followed by amplification creates a new template strand. Templates are then PCR amplified to add indexes, creating sequence-ready libraries.

Figure 2: MiSeq System

The MiSeq System leverages the latest advances in SBS chemistry and the industry's simplest workflow.

^{*} Assays are defined as the probe set measuring the expression of a single region of interest.

Flexible Options with Fixed Panels or Custom Content

TruSeq Targeted RNA Expression Library Preparation Kits are available with fixed or custom panels. For disease-focused expression studies, fixed panels offer pre-designed, ready-to-use assays for commonly studied diseases, or molecular pathways (Table 1). Fixed content panels are ideal for profiling many samples at once, screening cell types quickly and economically, or validating microarray results.

Beyond the validated assays provided in fixed panels, content may be expanded on any panel by adding 12–1000 custom assays. Using DesignStudio™, a free, online tool accessible through Mylllumina, simply upload a list of targets and choose from over 540,000 pre-designed assays based on RefSeq gene models in human, rat, or mouse. By quickly building a custom panel, adding new targets to previously ordered custom panels, or choosing one of many fixed panels, the TruSeq Targeted RNA Expression Solution has the flexibility to meet specific research needs.

The flexible MiSeq System provides opportunities to switch easily from one sequencing application to another in pursuit of evolving research goals. Beyond RNA profiling, the MiSeq System is fully compatible with a broad range of research applications, including *de novo* sequencing, 16S metagenomics, small genome sequencing, amplicon sequencing, HLA Typing, and more. While the TruSeq Targeted RNA Expression application requires only 50 bp reads, the MiSeq System can produce 2 × 300 bp read lengths for additional applications.

Apoptosis	Neurodegeneration
Cardiotoxicity	NFkB Pathway
Cell Cycle	Stem Cell
Cytochrome P450	TP53 Pathway
Hedgehog Pathway	Wnt Pathway

Simple, Streamlined Data Analysis

Data analysis, including alignment, quantitation, and data visualization, takes place directly on the MiSeq instrument with MiSeq Reporter (Figure 3). Pairwise comparisons for relative expression between samples or groups of samples is simple and intuitive. Customizable significance thresholds allow quick identification of differentially expressed targets. From library preparation to final data analysis, the MiSeq Targeted RNA Expression Solution offers a simple, fully integrated workflow.



TruSeq Targeted RNA Expression and FFPE Samples

TruSeq Targeted RNA Expression panels have a robust design compatible with high to low-quality or low-quantity RNA. The small amplicon size and short read requirements allow successful target detection with degraded or poor-quality samples. For additional guidance using TruSeq Targeted RNA Expression Kits with formalin-fixed, paraffin-embedded (FFPE) or highly degraded samples, see the Expression Analysis of FFPE Samples Illumina Technical Note. The low 50 ng RNA input requirement enables TruSeq Targeted RNA Expression studies with precious samples or low-quantity samples, such as tumor RNA.

Summary

Designed for the MiSeq system, TruSeq Targeted RNA Expression provides accurate and efficient RNA profiling and validation for gene expression studies. Combine fixed panels focused on biological pathways or disease-specific markers with custom content for maximum flexibility. In less than two days, go from RNA sample to data with a simple, integrated workflow.

Learn More

For more on TruSeq Targeted RNA Expression, visit: www.illumina.com/products/truseq-targeted-rna-expression-kits.ilmr

To learn more about read budget and normalization with TruSeq Targeted RNA Expression Panels, visit:

www.illumina.com/documents/products/technotes/technote_truseq_targ_rna_design.pdf

References

- Illumina (2014) MiSeq system specification sheet. (www.illumina.com/documents/products/datasheets/datasheet_miseq.pdf)
- Nakazato T, Ohta T, Bono H. (2013) Experimental design-based functional mining and characterization of high-throughput sequencing data in the sequence read archive. PLoS One 22;8(10): e77910.
- Ross MG, Russ C, Costello M, Hollinger A, Lennon NJ, et al. (2013)
 Characterizing and measuring bias in sequence data. Gen Biol 14: R51.
- Liu L, Li Y, Li S, Hu N, He Y, et al. (2012) Comparison of next-generation sequencing systems. J Biomed Biotechnol 2012: 251364.
- Illumina (2014) Expression analysis of FFPE samples. (www.illumina.com/ documents/products/technotes/technote-expression-analysis-ffpe-samples. pdf)

Product Specifications

Specification	Value
Database content	> 540,000 designs
Database sontone	(mouse, human, rat)
Target types	Gene, transcript, exon,
	splice junction, cSNP, fusion
Dynamic range	5 orders of magnitude
Time to answer	1.5 days
Hands-on time	4 hours
RNA quality	> 200 bp unfixed or FFPE

Join the Illumina Community

With a MiSeq System in their laboratory, researchers join a worldwide community of over 100,000 scientists using Illumina technology for their research studies. Illumina schedules community events throughout the year, bringing researchers together to share ideas. User group meetings, scientific symposiums, and blog forums provide venues to discuss new research methods and breakthrough studies.

An integral part of the Illumina community is our dedicated service and support team, consisting of more than 300 people worldwide, 75% of whom have advanced degrees. Illumina technical support begins when the MiSeq System is delivered, with Illumina scientists and engineers assisting with system installation and setup, and the training of laboratory personnel. They are there 24/7 globally to answer questions every step of the way, giving researchers the peace of mind to focus on their next research study.

As researchers' needs change, new systems are brought into the laboratory, or new methods are undertaken, the Illumina support and training teams are there to provide assistance. In addition to on-site support, training courses (via webinar or at an Illumina facility) are available to bring laboratory personnel quickly up to speed.

Ordering Information

Product Name	Number of Samples	Catalog No.
TruSeq Targeted RNA Expression Custom Components		
TruSeq Targeted RNA Custom Kit	48	RT-101-1001
	96	RT-102-1001
TruSeq Targeted RNA Supplemental Content	48	RT-801-1001
	96	RT-802-1001
TruSeq Targeted RNA Expression Fixed Panels		
TruSeq Targeted RNA Apoptosis Panel Kit	48	RT-201-1010
	96	RT-202-1010
TruSeq Targeted RNA Cardiotoxicity Panel Kit	48	RT-201-1009
	96	RT-202-1009
TruSeq Targeted RNA Cell Cycle Panel Kit	48	RT-201-1003
	96	RT-202-1003
	48	RT-201-1006
TruSeq Targeted RNA Cytochrome p450 Panel Kit	96	RT-202-1006
	48	RT-201-1002
TruSeq Targeted RNA Hedgehog Panel Kit	96	RT-202-1002
	48	RT-201-1001
TruSeq Targeted RNA Neurodegeneration Panel Kit	96	RT-202-1001
	48	RT-201-1008
TruSeq Targeted RNA NFkB Panel Kit	96	RT-202-1008
TruSeq Targeted RNA Stem Cell Panel Kit	48	RT-201-1005
	96	RT-202-1005
	48	RT-201-1007
TruSeq Targeted RNA TP53 Pathway Panel Kit	96	RT-202-1007
	48	RT-201-1004
TruSeq Targeted RNA Wnt Pathway Panel Kit	96	RT-202-1004
TruSeq Targeted RNA Expression Index Kits		
TruSeq Targeted RNA Index Kit	48	RT-401-1001
TruSeq Targeted RNA Index Kit A	96	RT-402-1001
TruSeq Targeted RNA Index Kit B	96	RT-402-1002
TruSeq Targeted RNA Index Kit C	96	RT-402-1003
TruSeq Targeted RNA Index Kit D	96	RT-402-1004

Illumina • 1.800.809.4566 toll-free (U.S.) • +1.858.202.4566 tel • techsupport@illumina.com • www.illumina.com

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