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Targeted RNA Expression Solutions on the MiniSeq™ System

The MiniSeq System delivers a clear, cost-effective, and customizable solution for quantified targeted RNA expression analysis

Highlights

- **Rapid Multiplexed Targeted RNA Sequencing**
  Users can analyze 12–1000 targets per sample and up to 384 samples in a single sequencing run
- **Accessible, Affordable NGS Technology**
  Access a personal, reliable sequencing platform for low-throughput RNA sequencing solutions
- **Fixed or Custom Targeted Content**
  Flexible solution allows fixed or customized sequencing panels as well as add-on content
- **Exceptional Data Quality**
  Build confidence in your results with the most widely adopted, proven Illumina SBS chemistry[1]

Figure 1: Targeted RNA Sequencing—Targeted RNA-Seq on the MiniSeq System allows cost-effective differential expression profiling and identification of gene fusions, splice junctions, and cSNPs.

Introduction

Targeted RNA sequencing (RNA-Seq) is a highly accurate method for selecting and sequencing-specific transcripts of interest (Figure 1). The MiniSeq Targeted RNA Sequencing workflow offers an affordable and simple next-generation sequencing (NGS) solution for custom or preconfigured targeted RNA-Seq studies (Figure 2). Targeted RNA sequencing allows differential expression analysis, allele-specific expression measurement, and the identification of gene fusions, splice junctions, and cSNPs. With qualitative and quantitative RNA-Seq data, you can obtain data across a large dynamic range, providing higher sensitivity and accuracy compared to conventional expression analysis methods.

Simple, Integrated Workflow

Library Preparation

The MiniSeq System Targeted RNA-Seq solution delivers a complete sample-to-answer workflow for analyzing custom or fixed sequencing panels (Figure 2). The targeted RNA-Seq workflow begins with library preparation using TruSeq® Targeted RNA Expression Kits (Figure 3). The TruSeq Kits offer highly customizable mid- to high-plex gene expression profiling and validation for the MiniSeq System. With the TruSeq Targeted RNA Expression Kits, there are 3 options for targeted content 1) fixed sequencing panels 2) custom sequencing panels and 3) custom content as an add-on to fixed panels.

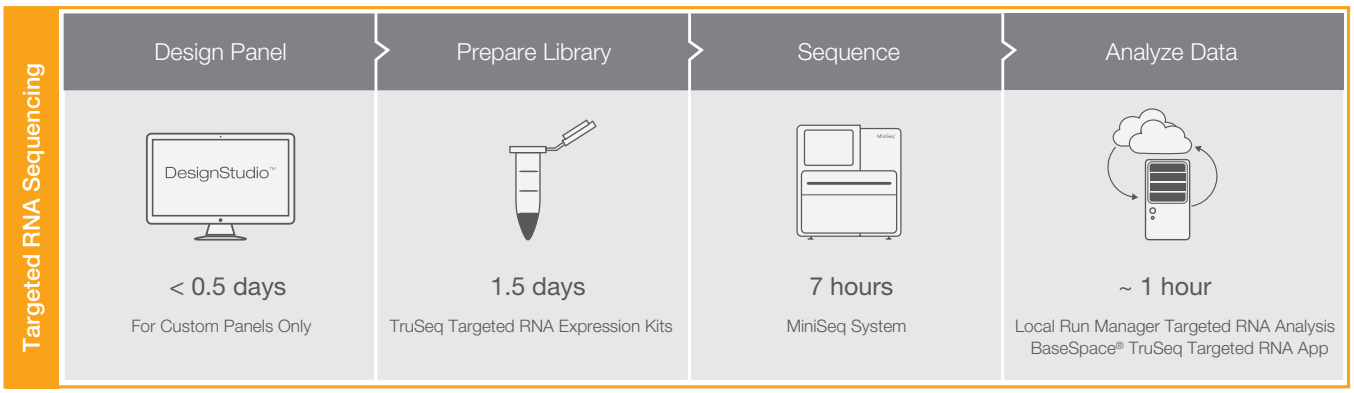

Figure 2: MiniSeq System Targeted RNA-Seq Workflow—The integrated workflow enables streamlined library preparation, sequencing, and data analysis, allowing cost-effective studies for a broad range of RNA samples.

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Advantages of Targeted RNA-Seq Over Microarrays

- Provides *qualitative* and *quantitative* data
- Enables deep sequencing for greater sensitivity and dynamic range
- Measures dozens to thousands of targets simultaneously
- Enables superior analysis of low-quality or FFPE-derived RNA
- Delivers a better value, with lower price/sample than many gene expression arrays

Starting with as little as 50 ng of RNA, the TruSeq Targeted RNA Assay is part of a simple workflow with convenient online panel design and ordering, and automated, on-instrument data analysis. TheTruSeq Targeted RNA Kits are also designed to accommodate a broad range of sample types including low-quality or formalin-fixed, paraffin-embedded (FFPE) samples.

Fixed Sequencing Panels

Fixed, or preconfigured TruSeq Targeted RNA-Seq Panels provide the convenience required to quickly start and complete projects, while delivering the quality needed from expert-defined content. The TruSeq Targeted RNA Expression Panels focus on specific cellular pathways or disease processes, including:

- Apoptosis
- Cardiotoxicity
- Cell Cycle
- Hedgehog Pathway
- Neurodegeneration
- NFkB Pathway
- P450 Pathway
- P53 Pathway
- Stem Cells
- Wnt Pathway

For enhanced flexibility, custom content can be added to any TruSeq Targeted RNA Panel.

Custom Sequencing Panels and DesignStudio™

For specific regions of interest, researchers can design and order custom panels with DesignStudio, an easy-to-use, web-based, assay design tool. To get started with DesignStudio, simply upload a list of targets identified by RNA-Seq or microarray experiments or choose from over 400,000 predesigned assays based on RefSeq gene models in human, rat, and mouse. Quickly build a custom panel with up to 1000 assays or add new targets to a previously ordered panel. DesignStudio provides dynamic feedback to optimize target region coverage, reducing the time required to design custom projects.

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 are preloaded with all required sequencing reagents. The 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.

TruSeq Targeted RNA Expression Chemistry

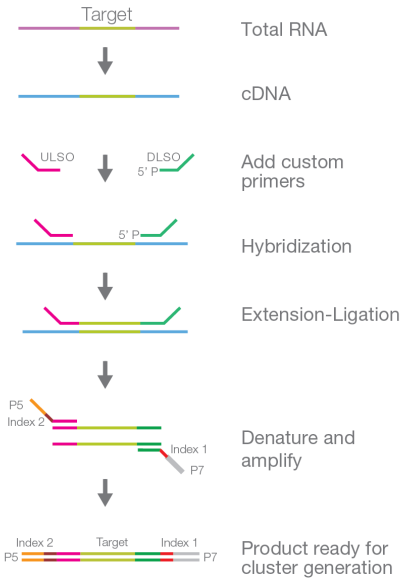

Figure 3: TruSeq Targeted RNA Library Preparation Chemistry—TruSeq Targeted RNA Library Prep Kits provide a simple, cost- effective method for generating targeted RNA libraries directly from total RNA.

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

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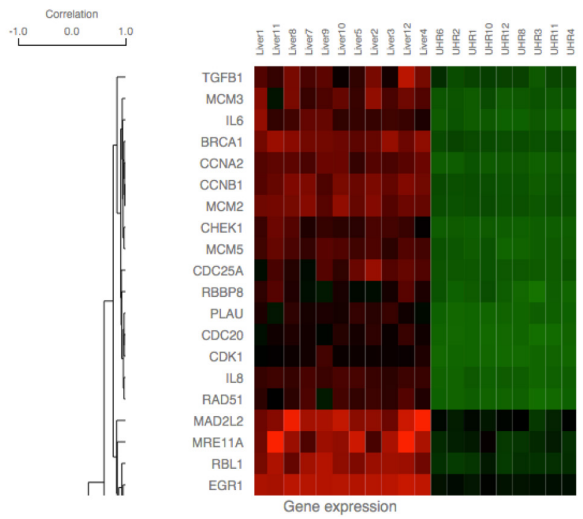

Figure 5: Targeted RNA-Seq Analysis in BaseSpace Cloud—View clustered heat maps with the TruSeq Targeted RNA v1.0 App in BaseSpace.

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. 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. These Apps deliver optimized analysis pipelines that support a range of common RNA-Seq data analysis needs such as alignment, variant calling, and more. The TruSeq Targeted RNA v1.0 App[2] aligns targeted sequence reads using a banded Smith-Waterman alignment, quantifies the relative expression of genes and isoforms between several samples, and compares abundance across input samples (Figure 5).

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.

Expression Analysis of FFPE Samples

RNA isolated from FFPE samples can vary in quality among different specimens, or within different samples from the same specimen. These samples can prove challenging for conventional expression analysis methods such as RT-PCR or microarray analysis. TruSeq

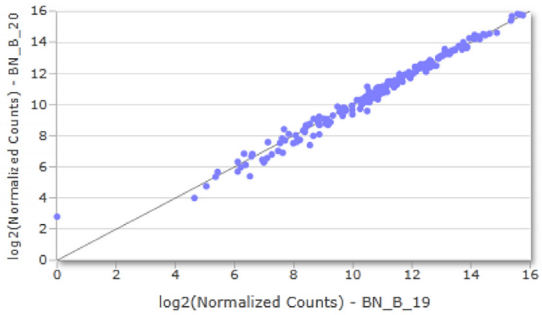

Figure 6: Targeted Sequencing with FFPE Samples—Gene expression profiles for FFPE breast cancer samples prepared with the TruSeq Targeted RNA expression kit. $R^2 = 0.987$.

Targeted RNA Expression Kits offer an accurate and powerful method for analyzing low-quality or FFPE samples (Figure 6). With the ability to profile 12–2000 targets per sample, and to multiplex up to 384 samples per run, the TruSeq Targeted RNA Expression Kits enables efficient and cost-effective analysis of common FFPE tissues such as tumor samples or tumor-normal paired samples.

Targeted RNA-Seq vs Expression Microarrays

Compared to microarrays, RT-PCR, and other traditional technologies, NGS targeted RNA-Seq offers several advantages. Targeted RNA-Seq allows deep sequencing, which provides higher sensitivity and a virtually unlimited dynamic range. This sensitivity enables superior performance for the detection and analysis of low-abundance, low-quality, or FFPE samples. 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 Targeted RNA Sequencing Solution offers a highly sensitive and accurate method for analyzing specific transcripts of interest. By harnessing the broad dynamic range of NGS sequencing, researchers can obtain more sensitive and accurate measurements for their targeted gene expression studies. Whether looking for the speed of a fixed panel or the flexibility of a custom panel, the MiniSeq Targeted RNA Sequencing Solution delivers high-quality NGS data in a smaller, more accessible platform.

Learn More

For more on DesignStudio and Targeted RNA Expression Experiments, go to www.illumina.com/content/dam/illumina-marketing/documents/products/technotes/truseq_targ_rna_design.pdf.

To learn more about expression analysis of FFPE samples, visit: www.illumina.com/content/dam/illumina-marketing/documents/products/technotes/technote-expression-analysis-ffpe-samples.pdf.

For more on the TruSeq Targeted RNA Expression fixed panels, go to: www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/datasheet_truseq_targeted_rna_expression.pdf.

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References

1. Nakazato T, Ohta T, Bono H. Experimental design-based functional mining and characterization of high-throughput sequencing data in the sequence read archive. *PLoS One.* 2013;22;8(10): e77910.
2. TruSeq Targeted RNA v1.0 App (www.illumina.com/informatics/research/sequencing-data-analysis-management/basespace/basespace-apps/truseq-targeted-rna-1386385.html). Accessed 15 December 2015.

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

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

Ordering Information

Sequencing System	Catalog No.
MiniSeq System	SY-420-1001
Sequencing Kits	
MiniSeq High Output Kit (75 Cycles)	FC-420-1001
MiniSeq High Output Kit (150 Cycles)	FC-420-1002
MiniSeq High Output Kit (300 Cycles)	FC-420-1003
MiniSeq Mid Output Kit (300 Cycles)	FC-420-1004

TruSeq Targeted RNA Expression Custom Components	Sample No.	Catalog No.
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 and TruSight 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
TruSeq Targeted RNA Cytochrome p450 Panel Kit	48	RT-201-1006
	96	RT-202-1006
TruSeq Targeted RNA Hedgehog Panel Kit	48	RT-201-1002
	96	RT-202-1002
TruSeq Targeted RNA Neurodegeneration Panel Kit	48	RT-201-1001
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TruSeq Targeted RNA NFκB Panel Kit	48	RT-201-1008
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TruSeq Targeted RNA Stem Cell Panel Kit	48	RT-201-1005
	96	RT-202-1005
TruSeq Targeted RNA TP53 Pathway Panel Kit	48	RT-201-1007
	96	RT-202-1007
TruSeq Targeted RNA Wnt Pathway Panel Kit	48	RT-201-1004
	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

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