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TruSeq® Targeted RNA Expression

Highly customizable and affordable mid-plex gene expression analysis for the MiSeq® system.

Highlights

- **Content and flexibility with fixed and customizable panels**
  Choose validated pathway, cell, or disease-specific fixed panels, or add customized content
- **Mid-plex gene expression at a complexity and scale not previously possible**
  Examine 1,000 targets per sample, 384 samples per run
- **Fast and simple workflow**
  Go from RNA to data in less than two days

Introduction

TruSeq Targeted RNA Expression leverages proven MiSeq sequencing technology to deliver an accurate and powerful method for validating gene expression arrays and RNA-Seq studies. TruSeq Targeted RNA Expression (Figure 1) enables efficient, quantitative multiplexed gene expression profiling for 12-1,000 targets per sample and up to 384 samples in a single MiSeq run. Requiring just 50 ng or less of starting RNA, TruSeq Targeted RNA Expression is amenable to a wide range of samples. Choose from over 400,000 pre-designed assays to create a custom panel targeting genes, exons, splice junctions, cSNPs and fusions. Fixed panels offer a wide variety of biological pathways and disease-specific markers, or combine fixed and custom content for the ultimate in flexibility. TruSeq Targeted RNA Expression offers a fully integrated solution, including convenient online assay design and ordering, a streamlined workflow, and automated, on-instrument data analysis.

Choose Fixed Panels for Focused Studies

For pathway- or disease-focused expression or profiling studies, TruSeq Targeted RNA Expression fixed panels offer ready-to-use assays designed for commonly studied genes (Table 1). Validated, fixed content panels are ideal for profiling many samples or screening cell types quickly and economically, and providing base content that can be expanded upon with custom content as needed.

Increase Your Flexibility with Custom Content

TruSeq Targeted RNA Expression assays are pre-designed assays targeting exon junctions and non-junction sites, as well as target SNPs within coding regions. Choose validated assays in DesignStudio™, a free, user-friendly tool accessed through your MyIllumina account[1].

Figure 1: TruSeq Targeted RNA Expression

TruSeq Targeted RNA Expression delivers fixed or customizable affordable mid-plex gene expression that takes full advantage of the throughput and flexibility of the MiSeq® system.

Create fully custom panels of 12–1,000 assays, or add specific genes or regions to one of the fixed panels, or to a previously ordered custom panel. Simply select the assays you need and add them to your order, with no design time.

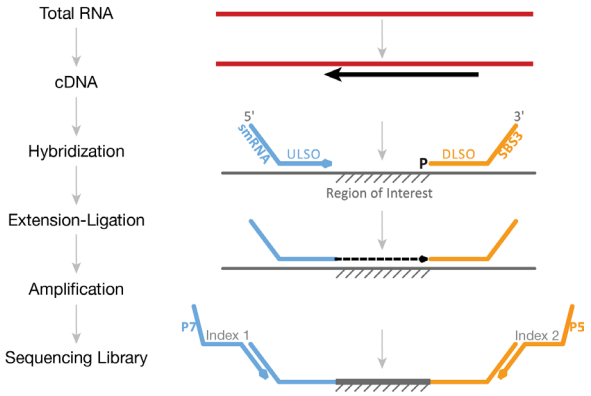
Streamlined, Targeted Assay Workflow

TruSeq Targeted RNA Expression for custom or fixed designs features a simple method for generating indexed, sequence-ready libraries from RNA regions of interest (Figure 2). Starting with as little as 50 ng of total RNA, the small amplicon size allows successful target detection, even on poor quality samples. All targets are amplified in a single reaction, minimizing potential bias and workflow steps compared to methods such as qPCR. From sample to data analysis, the entire process takes less than two days.

Table 1: TruSeq RNA Expression Fixed Panels

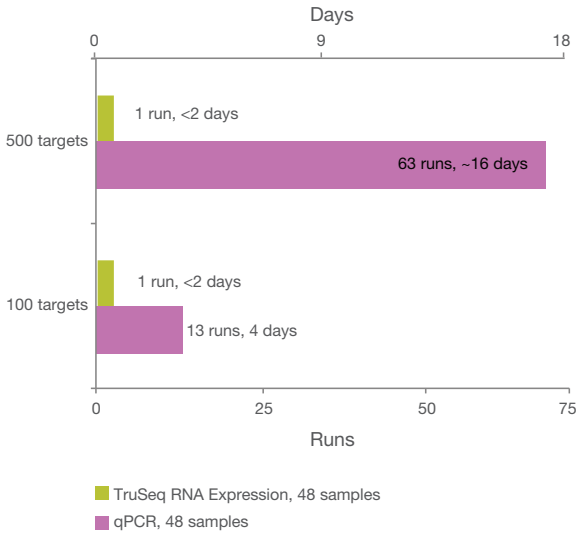
Apoptosis	Hedgehog Pathway	TP53 Pathway
Cardiotoxicity	Neurodegeneration	Wnt Pathway
Cell Cycle	NFκB Pathway	
Cytochrome P450	Stem Cell	

Figure 2: TruSeq Targeted RNA Expression Workflow



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 indices, creating sequence-ready libraries.

Figure 3: TruSeq Targeted RNA vs qPCR Workflow



With TruSeq RNA Expression, run 500 targets on 48 samples in one run in less than two days, compared to 63 runs in ~16 days with qPCR methods.

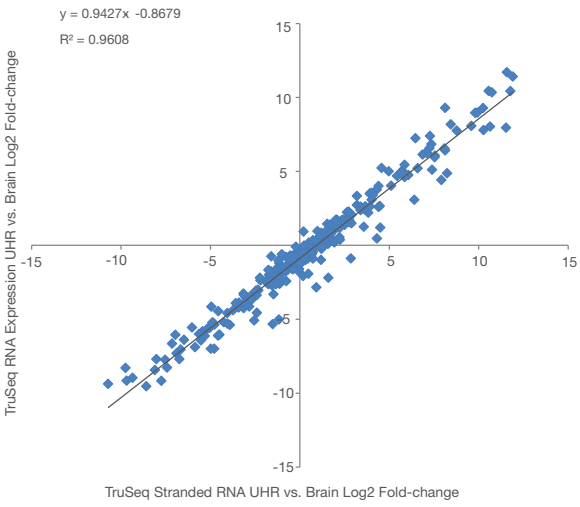
Multiplexing at a Scale not Previously Possible

With TruSeq Targeted RNA Expression, you can run up to 384 dual-index combinations to efficiently multiplex samples within a single MiSeq run. With 25 million reads, the MiSeq system is capable of generating 25,000 datapoints per run (at an average of 1,000 reads per target), equivalent to 65 384-well plates. Compared to qPCR, the number of runs and amount of processing time is significantly decreased (Figure 3). For more information about read budget, normalization, and getting the best results from your TruSeq Targeted RNA Expression assays, refer to the technical note[2].

Accurate Confirmation Using TruSeq Targeted RNA Expression

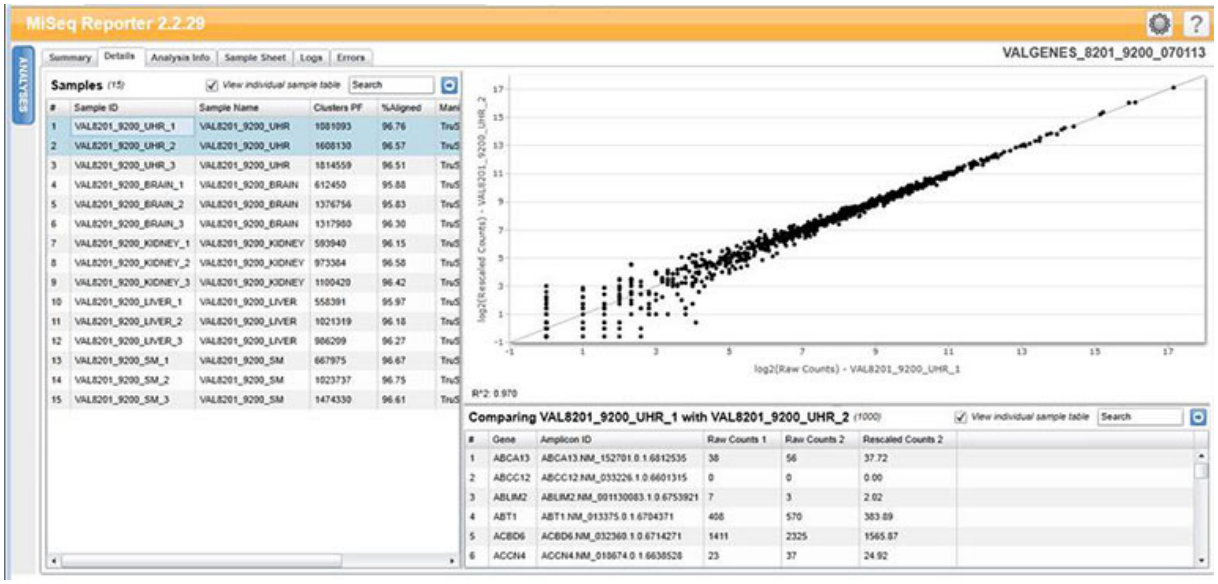
TruSeq Targeted RNA Expression was compared against the gold standard RNA-Seq for fold-change in an experimental target set. As shown in Figure 4, fold-change expression in 281 targets between Universal Human Reference (UHR) RNA and total brain mRNA was measured using TruSeq Targeted RNA Expression (X-axis) and TruSeq Stranded RNA-Seq (Y-axis). Data show excellent correlation, demonstrating that TruSeq Targeted RNA Expression provides accurate validation. The assay is also highly reproducible, even over a large dynamic range (Figure 5).

Figure 4: Fold-Change Correlation between RNA-Seq and TruSeq Targeted RNA Expression



Comparison of fold change expression between Universal Human Reference (UHR) and brain mRNAs for 281 targets, using TruSeq Stranded RNA-Seq (X-axis) and TruSeq RNA Expression (Y-axis).

Figure 5: Visualization of TruSeq Targeted RNA Expression Data using MiSeq Reporter



Data visualization with MiSeq Reporter allows easy comparison of data sets.

Product Specifications

Specification	Value
Database content	> 400,000 designs (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

Simple Data Analysis

After a sequencing run on the MiSeq system, data are automatically aligned and can be viewed using the MiSeq Reporter. As shown in Figure 5, pairwise comparisons for relative expression between samples or groups of samples is simple and intuitive. Customizable significance thresholds allow you to quickly identify differentially expressed targets. The TruSeq Targeted RNA Expression user experience is customized and streamlined, and keeps project data highly accessible.

Summary

Designed for the MiSeq system, TruSeq Targeted RNA Expression provides rapid and economical RNA profiling and validation for your gene expression studies. Go from sample to answer in less than two days with a simple, streamlined workflow and automated data visualization. Choose validated, pre-designed panels or add custom content to your existing assays for the ultimate flexibility to evolve your research.

References

1. https://icom.illumina.com/
2. Considerations for Designing a Successful TruSeq Targeted RNA Expression Experiment Technical Note, 2013.

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
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
	96	RT-202-1001
TruSeq Targeted RNA NFκB Panel Kit	48	RT-201-1008
	96	RT-202-1008
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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