DATA SHEET

Illumina Stranded mRNA Prep, Ligation

A fast, flexible solution for highly accurate analysis of the coding transcriptome

- Achieve high-quality data and enhanced gene discovery from as little as 25 ng RNA
- Prepare libraries in 7 hours with < 3 hours of hands-on time
- Multiplex up to 384 samples in a single run with unique dual indexes

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Introduction

RNA sequencing (RNA-Seq) with next-generation sequencing (NGS) is a powerful method for discovering, profiling, and quantifying RNA transcripts. Key RNA-Seq approaches include:

- Messenger RNA (mRNA)-Seq sensitively and accurately quantifies gene expression, identifies known and novel isoforms in the coding transcriptome, and measures allele-specific expression
- Total RNA-Seq provides an unbiased, hypothesis-free approach for comprehensive analysis of the transcriptome. It accurately measures gene and transcript abundance and detects both known and novel features in coding and multiple forms of noncoding RNA
- Targeted RNA-Seq analyzes gene expression in a focused set of genes of interest. Targeted RNA-Seq via enrichment enables cost-effective RNA exome analysis using sequence-specific capture of the coding regions of the transcriptome. It is ideal for low-quality, formalin-fixed paraffin-embedded (FFPE) samples

TruSeq™ Stranded mRNA provides a robust solution for gene expression analysis and discovery applications in the coding transcriptome. However, a relatively high input requirement, long total assay time, and long hands-on time have limited its utility in RNA-Seq applications. To overcome these challenges, Illumina developed Illumina Stranded mRNA Prep. This advanced solution offers streamlined, rapid, ligation-based library preparation (Figure 1) that supports low sample inputs (Table 1) and a wide range of mRNA-Seq applications.

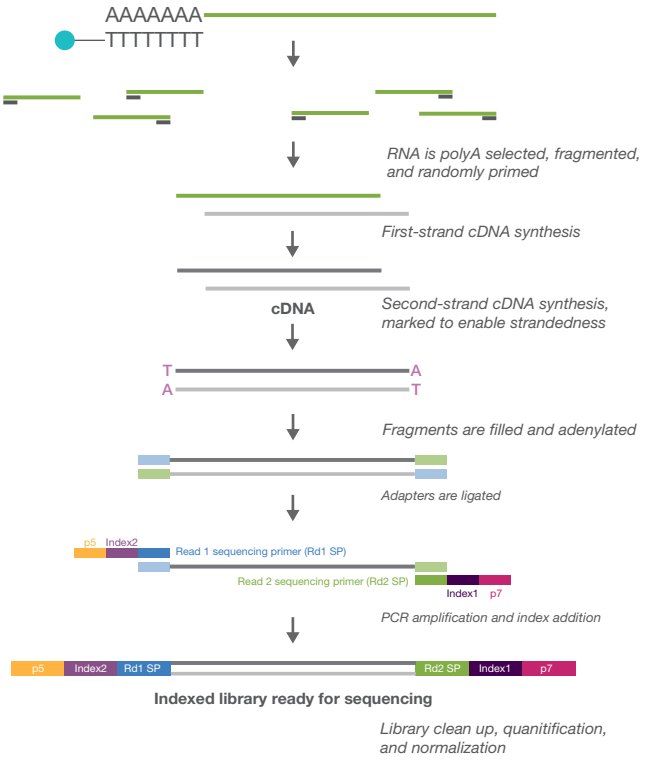

Figure 1: Illumina Stranded mRNA Prep—After polyA selection and cDNA synthesis is complete, ligation of unique dual index adapters and PCR amplification produces high-quality libraries that are quantified and normalized prior to sequencing.

Table 1: Illumina Stranded mRNA Prep specifications

Feature	TruSeq Stranded mRNA Prep	Illumina Stranded mRNA Prep
Max UDI	96	384
RNA input amount	100–1000 ng	25–1000 ng
Total assay time	10.5 hours	6.5 hours
Hands-on time	< 7 hours	< 3 hours
Kit configuration	48 or 96 samples	16 or 96 samples

UDI, unique dual indexes

High-quality data

Gene expression

Illumina Stranded mRNA Prep produces sequencing libraries from low input amounts that result in high-quality metrics for gene expression analysis (Table 2, Figure 2). Together, these results demonstrate the exceptional performance of Illumina Stranded mRNA Prep for gene expression applications. mRNA-Seq offers numerous advantages over non-NGS methods, including:

- Hypothesis-free experimental design, requiring no previous knowledge of the transcriptome
- Higher discovery power to detect known and novel transcripts
- Higher throughput capability to quantify hundreds to thousands of regions in each assay
- Broader dynamic range, providing more accurate measurement of gene expression
- More data per assay, providing full sequence and variant information

Table 2: Performance metrics for Illumina Stranded mRNA Prep, Ligation

Metric	100 ng RNA input		25 ng RNA input	
	TruSeq Stranded mRNA	Illumina Stranded mRNA Prep	TruSeq Stranded mRNA	Illumina Stranded mRNA Prep
% rRNA (28S/18S)	5.1	1.8	5.3	1.5
Strandedness	99.6	99.4	99.6	99.4
Median CV of coverage	0.49	0.46	0.50	0.47
% Duplicates	5.4	3.7	8.5	3.3
% Aligned	97.1	97.8	96.8	97.8

Results are from RNA Seq Alignment App v2.0.1 at 30M reads. Duplicates are reported at 4M subsampled pass-filter paired-end reads.

Gene discovery efficiency

To compare the performance of Illumina Stranded mRNA Prep to TruSeq Stranded mRNA for gene discovery applications, varying amounts of universal human reference (UHR) RNA were sequenced at 30M reads and the number of genes with 1× and 10× coverage was assessed. Results show that Illumina Stranded mRNA Prep enables greater gene detection, especially at low input amounts (Figure 3).

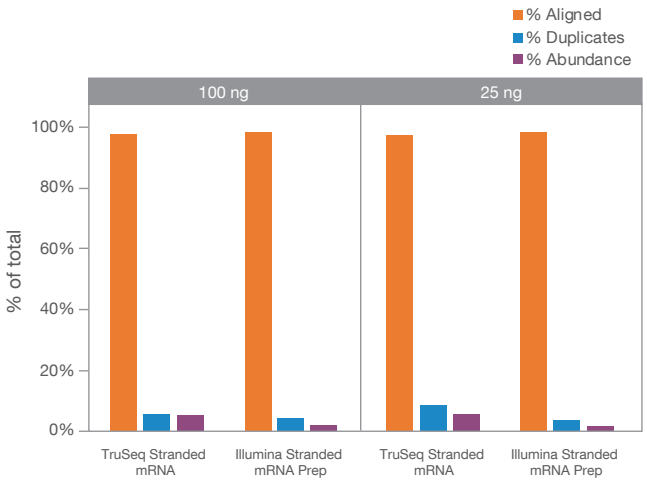

Figure 2: Comparison of performance metrics—Illumina Stranded mRNA Prep was compared against TruSeq Stranded mRNA. Illumina Stranded mRNA Prep showed superior performance, particularly with an input of 25 ng UHR RNA. Libraries were subsampled to 30M reads and analyzed using BaseSpace™ RNA-Seq Alignment App v2.0.1. Duplicates are reported at 4M subsampled paired-end reads passing filter.

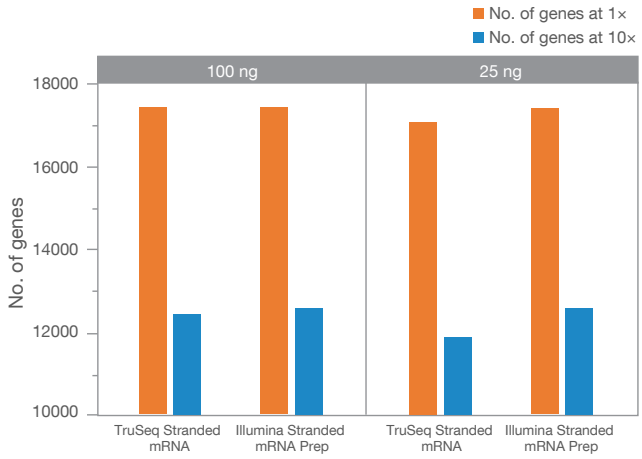

Figure 3: Greater gene discovery at low input—Illumina Stranded mRNA Prep enables greater gene detection with low RNA inputs, compared to TruSeq Stranded mRNA. The number of genes detected is reported at 30M subsampled paired-end reads PF. More genes detected at 1× coverage, as is the case with Illumina Stranded mRNA Prep, is an indicator of greater sensitivity.

Exceptional data concordance

Illumina Stranded mRNA Prep produces quality data with high concordance between technical replicates (Figure 4A) and varying amounts of input UHR RNA (Figure 4B). These results demonstrate that Illumina Stranded mRNA Prep is an ideal solution for samples with limited starting material. Also, Illumina Stranded mRNA Prep shows high data concordance with TruSeq Stranded mRNA, both with equivalent inputs (Figure 5A) and with reduced input (Figure 5B).

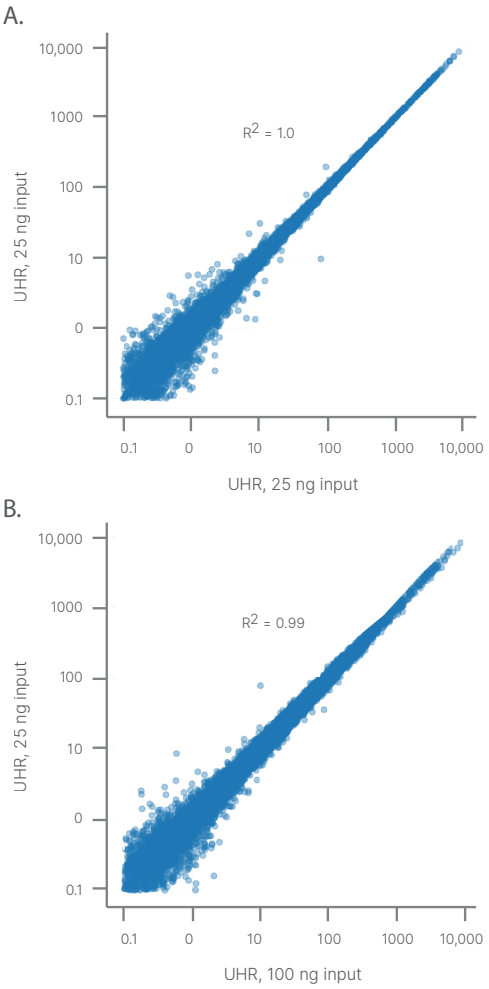

Figure 4: High data concordance—Illumina Stranded mRNA Prep achieves high data concordance between (A) technical replicates of 25 ng UHR RNA and (B) between input amounts of 25 ng and 100 ng UHR RNA. Libraries were sequenced at 2 × 74 bp, subsampled to 30M reads. Data analysis was performed using BaseSpace RNA-Seq Alignment App v2.0.1.

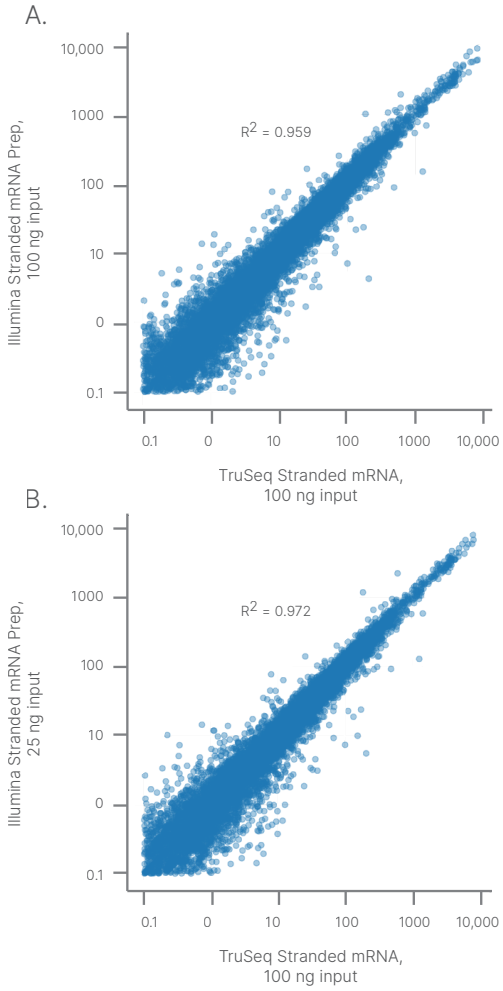

Figure 5: High concordance with legacy kit—Illumina Stranded mRNA Prep produces highly concordant data with TruSeq Stranded mRNA at (A) equivalent inputs of 100 ng UHR RNA and (B) with reduced input of 25 ng vs 100 ng UHR RNA.

Streamlined library preparation workflow

Illumina Stranded mRNA Prep uses a fast and flexible workflow for ligation-based preparation of RNA libraries (Figure 1). Innovations to the workflow, including shorter incubation times and reduced sample cleanup steps, result in a total assay time that is ~40% faster than TruSeq Stranded mRNA (Figure 6).

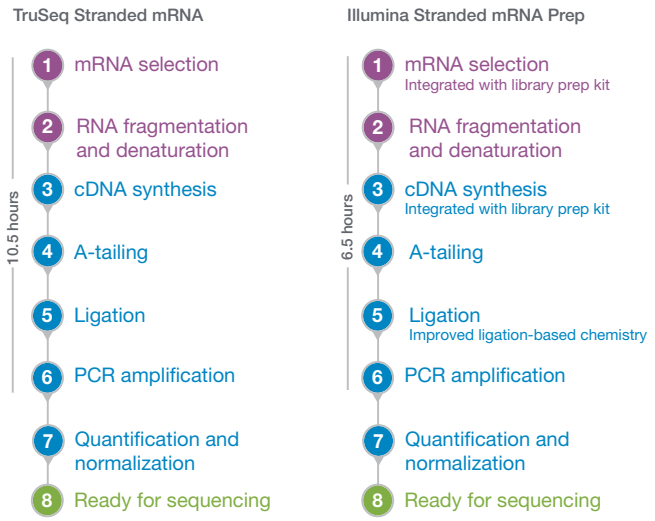

Figure 6: Illumina Stranded mRNA Prep workflow—Illumina Stranded mRNA Prep delivers a fast workflow with reduced hands-on time. Times may vary depending on equipment used, number of samples processed, automation procedures, or user experience.

Increased throughput with unique dual indexes

By combining Illumina Stranded mRNA Prep and high-throughput platforms, including the NextSeq™ 550 and NovaSeq™ 6000 Systems, laboratories can sequence significantly more samples per run without compromising data quality. For additional increases in sample throughput, Illumina Stranded Total RNA Prep supports multiplexing with 384 unique dual indexes (UDIs). In addition to eliminating the impact of index mis-assignment, or index hopping, UDIs help to decrease sequencing costs by allowing up to 384 samples to be loaded on a single NovaSeq 6000 S4 flow cell for significantly increased throughput.

Summary

Illumina Stranded mRNA Prep offers a streamlined solution for clear and comprehensive analysis across the coding transcriptome. This solution offers extraordinary flexibility for input type and supports low input amounts, down to 25 ng of high-quality RNA. Illumina Stranded mRNA Prep enables precise measurement of strand orientation, uniform coverage, and high-confidence discovery of features such as novel isoforms, gene fusions, and allele-specific expression.

Learn more

Illumina Stranded mRNA Prep, Ligation

Ordering information

Product	Catalog no.
Illumina Stranded mRNA Prep, Ligation (16 samples)	20040532
Illumina Stranded mRNA Prep, Ligation (96 samples)	20040534
Illumina RNA UD Indexes Set A, Ligation (96 indexes, 96 samples)	20091655
Illumina RNA UD Indexes Set B, Ligation (96 indexes, 96 samples)	20091657
Illumina RNA UD Indexes Set C, Ligation (96 indexes, 96 samples)	20091659
Illumina RNA UD Indexes Set D, Ligation (96 indexes, 96 samples)	20091661

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