Automating the Illumina TruSeq® Stranded mRNA Library Prep Protocol with the Tecan Freedom EVO® NGS Workstation*

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Introduction

RNA sequencing (RNA-Seq) is a powerful method for discovering, profiling, and quantifying RNA transcripts. Using Illumina next-generation sequencing technology, RNA-Seq does not require species- or transcript-specific probes, meaning the data are not biased by previous assumptions about the transcriptome. As the complexities of gene regulation have become better understood, a need for capturing additional data has emerged. Stranded information identifies the DNA strand from which a specific RNA transcript was derived, providing increased confidence in transcript annotation and visibility into antisense regulation.

This application note describes an automated Illumina-qualified† protocol developed for parallel processing of up to 48 samples using TruSeq Stranded mRNA kits with the Tecan Freedom EVO NGS workstation (Figure 1). The workstation's user-friendly TouchTools-based interface guides users through automation set up with minimal user intervention to deliver highly reproducible, sequencing-ready RNA-Seq libraries.

Experimental Design

Three input quantities (1 µg, 100 ng, and 500 ng) of total RNA were tested for this application note. A total of 24 libraries were prepared (four samples per RNA input quantity and type) using the same concentration of Illumina adapters for all three RNA amounts during the ligation step. All libraries underwent 15 cycles of PCR, followed by quality control using a LabChip GX (PerkinElmer #122000) instrument. The first 12 libraries were selected for pooling and paired-end, 2 x 50 bp sequencing across two lanes of a Rapid flow cell on the HiSeq® 2500 System.

Automated liquid handling steps for mRNA enrichment, first and second strand cDNA synthesis, adenylation, and adapter ligation were executed by the Tecan Freedom EVO NGS workstation.

Analysis and Results

Sequencing data were analyzed using BaseSpace® Core Apps for RNA Analysis[1] as recommended by Illumina. Libraries were generated successfully from all 24 samples, including those prepared from 100 ng total RNA input. Sequencing results from 12 representative samples showed a high percentage of reads mapped to the reference RNA. The percent median CV of coverage is consistent with manual library preparation methods, demonstrating equivalent coverage across the transcript. The correct strand orientation is preserved at > 99% (Table1). The data quality (Figures 2 and 3) indicates good correlation between same-sample types, demonstrating that the automated TruSeq Stranded mRNA protocol on the Tecan Freedom EVO NGS workstation provides a robust, walk-away workflow for library preparation.

Figure 1: Tecan Freedom EVO NGS Workstation

The Tecan Freedom EVO NGS workstation is set up to execute Illumina library preparation, including TruSeq Stranded mRNA. The system features the TouchTools operator interface for ease of use, three Inheco temperature controlled devices for thermal incubation and reagent cooling, and a heated shaker. A robotic manipulation arm and a plate magnet are provided for efficient bead clean-up steps. In addition, the compact worktable provides storage space for up to 12 tip boxes, allowing for longer unattended runs.

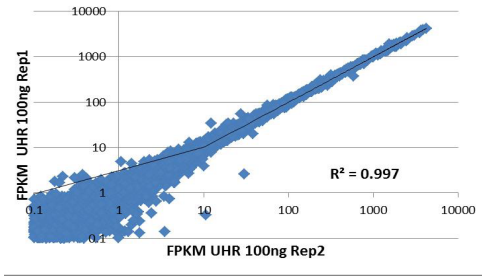
Conclusion

The automation-friendly workflow of the TruSeq Stranded mRNA protocol combined with the Tecan Freedom EVO NGS workstation provide a scalable and efficient solution for reproducible library preparation. The automated protocol can be completed with minimal hands-on time in just a day and a half, generating highly reproducible sequencing data the following day.

Learn More

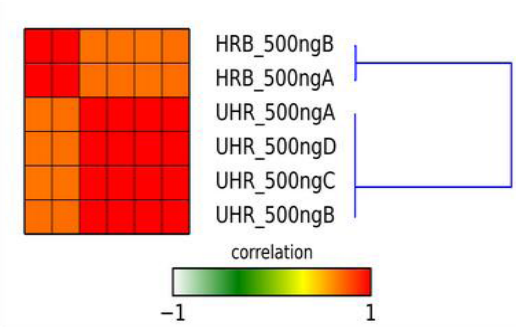
To obtain the TruSeq Stranded mRNA automation method for the Tecan Freedom EVO NGS workstation* discussed in this application note, visit www.tecan.com/ngs.

Figure 2: Technical Replicates Demonstrate Consistent Library Prep



Excellent correlation is shown for gene counts (FPKM) between technical replicates of Universal Human Reference (UHR) RNA, demonstrating consistent library preparation using the TruSeq Stranded mRNA automated workflow with the Tecan Freedom EVO NGS workstation.

Figure 3: Correlation in RNA Standards



UHR and HBR RNA samples cluster correctly, demonstrating no cross-contamination in libraries prepared using the TruSeq Stranded mRNA automated protocol with the Tecan Freedom EVO NGS workstation.

Table 1: Sequencing Data and Quality Metrics Summary

Sample[a]	Paired Reads	% Aligned	% Unaligned	% Human Ribosomal	% Adapter Dimer	% Median CV of Coverage	% Stranded
UHR_100ngA	15,142,715	96.9	3.1	1.3	0.0	54.0	98.9
UHR_100ngB	16,800,128	97.0	3.0	1.3	0.0	51.0	98.6
UHR_500ngA	31,641,184	97.2	2.8	0.8	0.0	56.0	99.3
UHR_500ngB	25,883,992	97.1	2.9	0.8	0.0	53.0	98.9
UHR_1µgA	33,395,488	97.1	2.9	0.6	0.0	52.0	99.2
UHR_1µgB	38,067,488	97.2	2.8	0.6	0.0	52.0	98.5
HRB_500ngA	21,390,901	97.2	2.8	0.3	0.0	56.0	99.1
HRB_500ngB	27,969,836	97.1	2.9	0.3	0.0	55.0	99.1
Average	**n/a**	**97.1**	**2.9**	**0.7**	**0.0**	**53.8**	**99.1**

Universal Human Reference (UHR) RNA (Agilent # 740000), and Human Brain Reference (HMR) RNA (Life Technologies # AM6050) were selected for qualification testing. The quality metrics demonstrate an average of 97% alignment and 99% correct strand orientation for libraries prepared with the TruSeq Stranded mRNA automated protocol using the Tecan Freedom EVO NGS workstation. Averages are represented by all 12 samples in the pool.

a. Data are shown for duplicate samples. Similar data were achieved for all 12 samples in the pool.

For questions regarding this application note, send inquiries to NGSPrep@tecan.com.

To learn more about TruSeq Stranded mRNA Library Prep kits, visit www.illumina.com/strandedmrna.

References

1. BaseSpace Core Apps for RNA Analysis (www.illumina.com/landing/basespace-core-apps-for-rna-sequencing/).

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