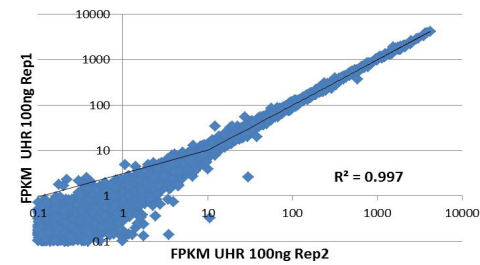
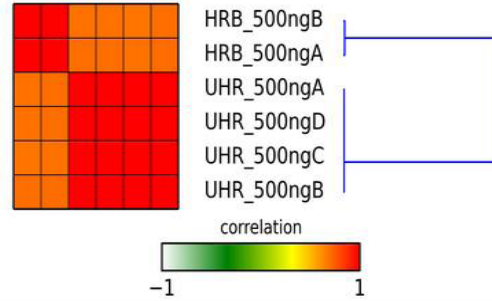


Figure 2: Technical Replicates Demonstrate Consistent Library Prep



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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† "Illumina-qualified" indicates that analysis by Illumina has shown that libraries prepared with the method perform comparably to those prepared manually.

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

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