Results

Juno system enables an automated, cost-effective approach to RNA sequencing

Figure 1. Samples and Advanta RNA-Seq reagents are added to the 48Atlas IFC, which is subsequently processed on the Juno instrument. The system solution automates many tedious hands-on steps to generate up to 48 RNA-seq libraries. The nanoscope design of the 48Atlas IFC significantly reduces reagent consumption, which helps minimize overall costs per sample.

New 48Atlas IFC format for solid-phase poly(A) RNA capture and multi-step reactions enabling walkaway automation

Figure 2. The 48Atlas IFC architecture automates multiple workflow steps otherwise performed manually, including poly(A) RNA capture, RNA fragmentation, reverse-transcription, sample-barcode PCR and multiple wash steps. The entire process can be completed in ~12.5 hr with only ~2 hr of hands-on-time. Following IFC barcode PCR, libraries are harvested, followed by purification and quantification prior to sequencing.

Table 1. Performance characteristics of the Advanta RNA-Seq Kit on Juno were assessed in an analytical validation study. The study was conducted using 3 Advanta reagent lots and 3 48Atlas IFC lots across 6 Juno instruments by 3 different operators. In total, more than 900 samples were sequenced comprising ~5 billion reads.

Gene and transcript detection across tissues with varying sample quality

Figure 3. Robust gene (3a) and transcript (3b) detection was observed from RNA extracted from samples of varying tissue origin and quality (RIN) using the Advanta RNA-Seq NGS Library Prep Kit. 100 ng was the input amount and samples were sequenced on an Illumina NextSeq™ instrument to an average read depth of ~20M reads per sample.

Percent of reads mapped to transcriptome (RefSeq) and average gene expression correlation between various input amounts

Figure 4. UHRR samples were sequenced with input amounts of 1 ng, 5 ng, 10 ng and 100 ng for a total of 64 samples. 10 ng to 100 ng were the recommended inputs amounts. Percent of reads mapped to the transcriptome (RefSeq) were calculated (4a) for the varying input amounts along with average pairwise correlations (4b) between inputs. This demonstrates no cliff effect below 10 ng, supporting excellent system robustness.

Differential transcript and exon expression between brain and UHRR samples for the gene GFAP

Figure 5. Read pile-ups for brain (5a) 10 ng (n = 4) and UHRR (5b) 10 ng (n = 4) samples processed with the Advanta Kit for the glial fibrillary acidic protein gene (GFAP). Corresponding reference transcripts plotted in panel 5c. As expected, little to no expression was observed in UHRR samples (relative to brain samples), consistent with its known expression in the central nervous system. The most likely isoform being expressed is shown in 5c, boxed in red. FASTQ files were first down-sampled to 30M reads per sample to equalize read depth.