Advanta RNA-Seq NGS Library Prep Kit

FLUIDIGM®

Automated nanoscale solution for RNA-Seq libraries

RNA sequencing (RNA-seq) is the gold standard for hypothesis-free profiling of the transcriptome and is an essential tool for molecular biology laboratories. To keep pace with increasing sample demand, automated microfluidics-based library prep can advance transcriptomics by streamlining laboratory methods and substantially reducing associated costs.

Designed to drive significant improvement in the RNA-seq workflow, the Advanta™ RNA-Seq NGS Library Prep Kit together with the Juno™ system provides an integrated solution for automated NGS library prep. The Juno system is easy to install and operate and, with the Advanta RNA-Seq reagents and 48.Atlas™ integrated fluidic circuit (IFC), supports simultaneous processing of up to 48 total RNA samples from eukaryotic organisms, generating libraries compatible with Illumina® sequencing instruments.

The method generates full-length, stranded libraries from random priming of polyadenylated [poly(A)] RNA present in total RNA samples. The Advanta RNA-Seq Kit and Juno workflow improve upon alternative RNA-seq library prep workflows by providing true walkaway automation while reducing reagent, consumables, labor and instrument costs.

Highlights

Walkaway automation— Substantially reduce pipetting steps and operator interventions using an automated 48-sample workflow that includes solid-phase capture of poly(A) RNA.

Superior cost savings— Maximize your laboratory budget by minimizing consumption of reagents and consumables using microfluidic technology.

Robust chemistry—Confidently generate high-quality full-length stranded RNA-seq libraries from a variety of organisms.

The Advanta RNA-Seq NGS Library Prep Kit and Juno system advantage

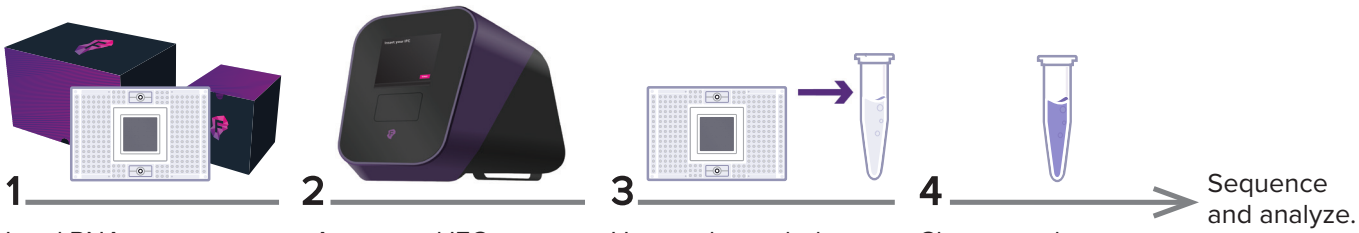

Figure 1. Advanta RNA-Seq NGS Library Prep Kit workflow on Juno. Samples and Advanta RNA-Seq reagents are added to the 48.Atlas IFC, which is subsequently processed on the Juno instrument. The system solution automates many tedious hands-on steps to generate up to 48 RNA-seq libraries. The nanoscale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

Advanta RNA-Seq workflow, 48.Atlas IFC and Juno enable significant efficiencies

The Advanta RNA-Seq Kit includes library preparation reagents, sample barcodes and the 48.Atlas IFC, a microfluidic device approximately the same size and shape as a standard 96-well plate. Sample and reagent mixes are dispensed into the IFC, which is then placed in the Juno system for automated processing of the majority of the workflow steps. As processing on Juno requires no manual intervention and no pipetting, the solution greatly reduces hands-on time as well as consumables cost and plastic waste. Nanoscale reaction volumes within the IFC significantly reduce reagent consumption for further meaningful cost savings.

48.Atlas IFC format enables walkaway automation

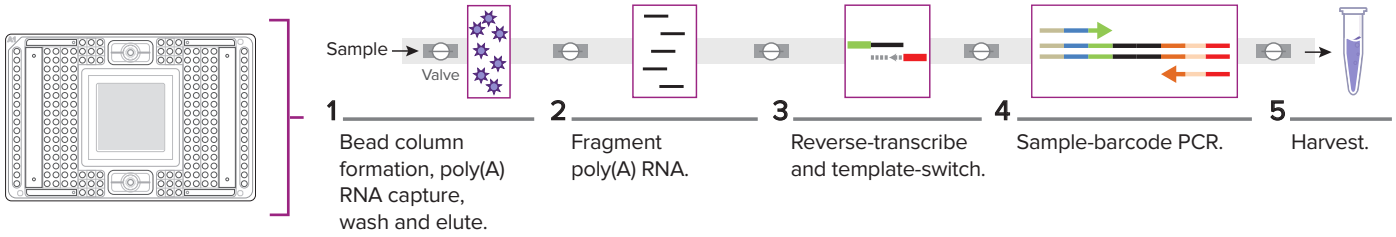

Figure 2. The 48.Atlas IFC architecture automates multiple workflow steps otherwise performed manually, including poly(A) RNA capture, RNA fragmentation, reverse-transcription, sample-barcode PCR and multiple wash steps.

Workflow comparison

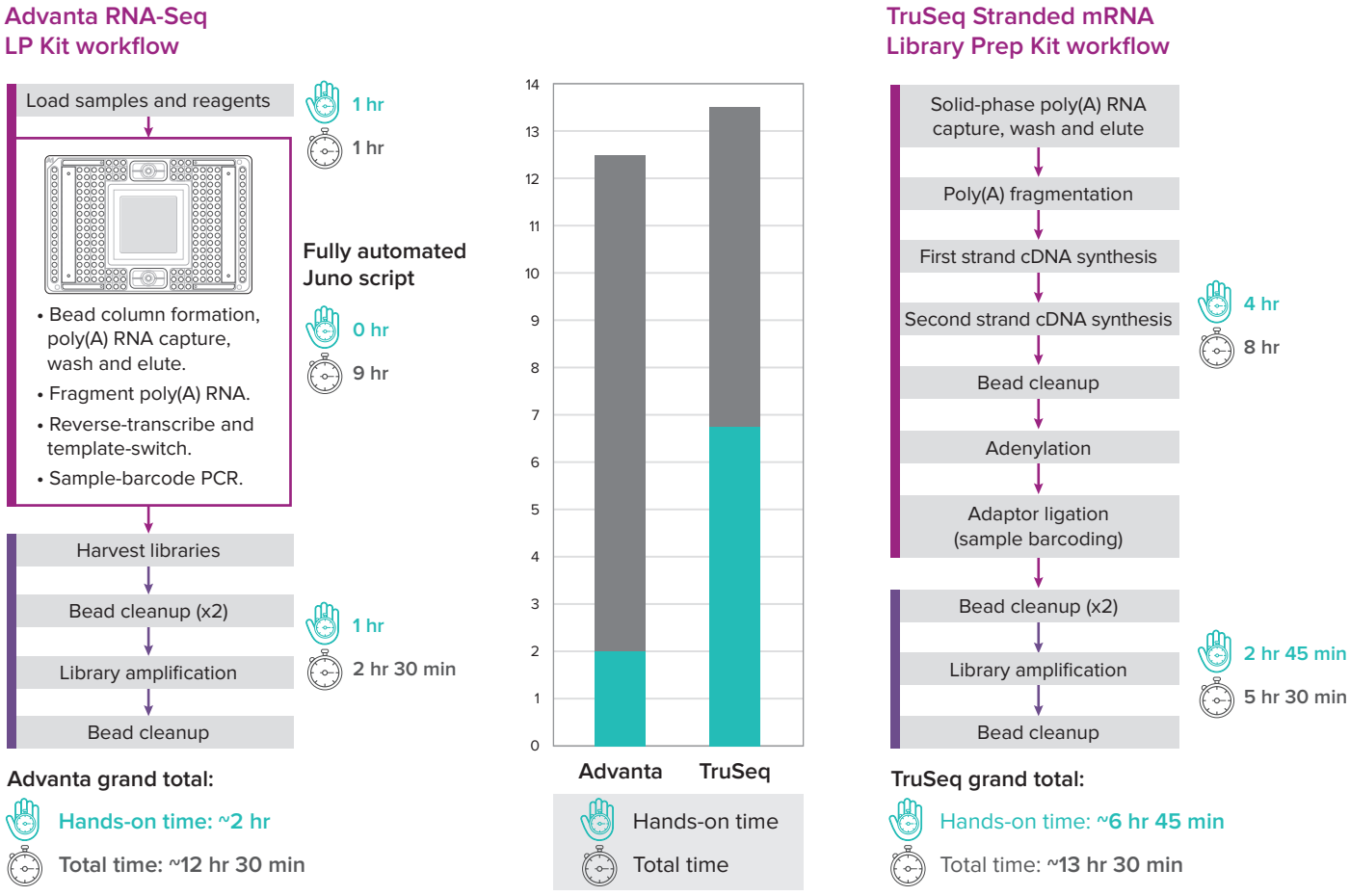

Figure 3. Example comparing hands-on and total time requirements for two RNA-seq workflows. Based on their respective protocols, the Advanta RNA-Seq NGS Library Prep Kit and TruSeq® Stranded mRNA Library Prep Kit (Illumina) are compared for processing 48 samples per batch. The Advanta RNA-Seq workflow using the 48.Atlas IFC with Juno system automation enable >70% hands-on time savings relative to the alternative TruSeq workflow.

Performance

Summary of performance results generated from analytical validation testing

Metrics	Human Total RNA Samples, Average				
	UHRR 10 ng	UHRR 100 ng	Brain 10 ng	Brain 100 ng	Overall
Percent reads mapped to genome (not including mtRNA and rRNA)	87.4%	86.8%	82.7%	80.1%	**84.3%**
Percent ribosomal RNA (rRNA) reads	3.0%	4.9%	5.4%	8.4%	**5.4%**
Percent unmapped reads	4.1%	3.8%	3.7%	4.1%	**4.0%**
Pearson's correlation of technical replicates within input amounts (10 ng vs 10 ng; 100 ng vs 100 ng)	0.984	0.992	0.980	0.992	**0.988**
Pearson's correlation of technical replicates between input amounts (10 ng vs 100 ng)	0.983		0.978		**0.981**
Percent correct strandedness	98.1%	98.6%	98.1%	98.5%	**98.3%**

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 48.Atlas IFC lots across 6 Juno instruments by 3 different operators. Samples were Universal Human Reference RNA (UHRR; Agilent®) and human adult brain RNA (Brain; BioChain®). In total, more than 900 samples were sequenced comprising ~5 billion reads.

Assessing technical reproducibility between two different operators

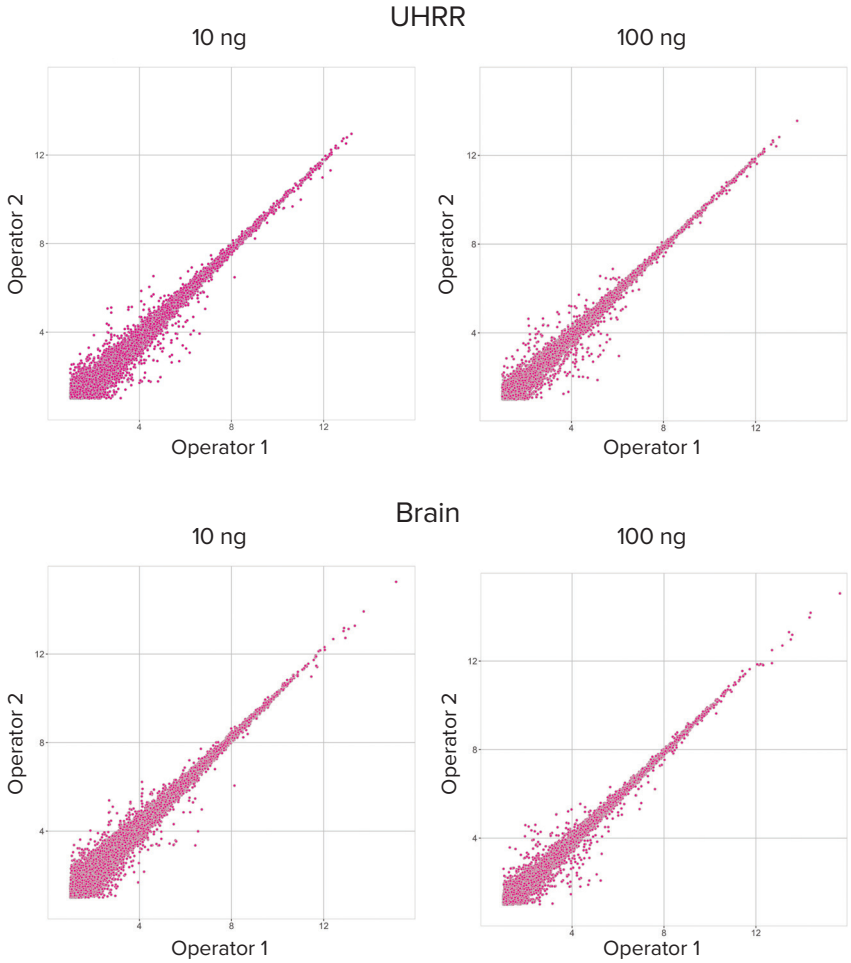

Figure 4. Gene-level Pearson's correlation between Operator 1 and Operator 2 using UHRR and Brain total RNA at 10 ng and 100 ng input amounts. On average, >99% concordance was observed, supporting excellent system robustness.

Assessing gene and transcript detection

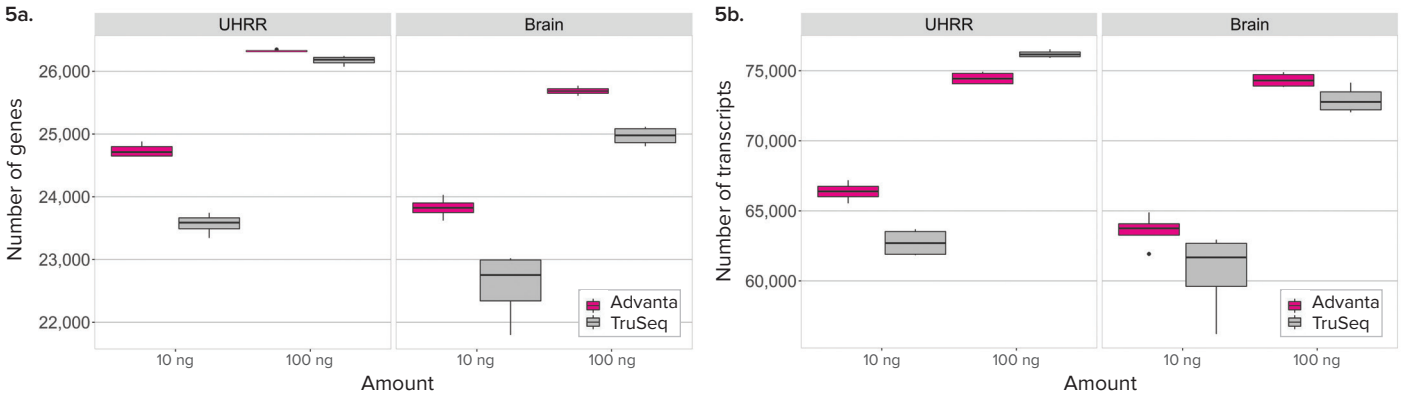

Figure 5. Comparison of gene (5a) and transcript (5b) detection per sample for libraries generated with Advanta RNA-Seq and TruSeq Stranded mRNA (Illumina) reagents. UHRR and Brain samples were processed with input amounts of 10 ng and 100 ng. Four replicates per condition were sequenced on a HiSeq 2500 using paired-end 2 x 75 bp. Samples were down-sampled to a read depth of 30M for all comparisons. (5a) Samples prepared with Advanta reagents consistently exhibited higher gene detection from both 10 ng and 100 ng starting sample amounts. (5b) Under most conditions, samples prepared with Advanta reagents exhibited higher transcript detection.

Product specifications

Attribute	Specification
Library type	Full-length, stranded libraries from random-primed poly(A) RNA
Sample input	Total RNA isolated from tissues and cell lines (RIN ≥7)
Input amount	10–100 ng
Library prep platform	Juno instrument with TX Interface Plate
Compatible IFCs	48.Atlas IFC
Samples per run per IFC	48 samples processed simultaneously per IFC
Assay time	Hands-on time ~2 hr; total turnaround time ~12 hr 30 min (from extracted RNA to libraries)
Samples per kit	192 (4 IFCs provided in kit)
Sample barcodes	96-sample barcoding using 12 (i7) x 8 (i5) dual indexes
Sequencer	Illumina® MiSeq™, NextSeq™, HiSeq® 2500, 3000, 4000 and X and NovaSeq™

Table 2. Overview of the Advanta RNA-Seq NGS Library Prep Kit specifications and attributes.

Product configuration

Advanta RNA-Seq NGS Library Prep Kit Materials included support 4 runs, 48 samples per run	**PN 101-9187** Quantity (boxes)
Advanta RNA-Seq NGS Library Kit reagent modules 1–4	4
48.Atlas IFCs	4
Control Line Fluid (150 µL each syringe)	1

Learn more at **fluidigm.com/advanta-rnaseq**

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