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Nanoliter-Scale Automation of Library Prep Solutions for RNA Sequencing

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Introduction

RNA sequencing (RNA-seq) provides a more precise measurement of the transcriptome than other expression profiling methods. We are developing an elegant microfluidics-based chemistry and workflow leveraging the Juno™ instrument.* Our RNA-seq method will support simultaneous processing of up to 48 samples with a one-click script on our system.

The Juno NGS system automates the RNA-seq workflow along with a new, nanoscale integrated fluidic circuit (IFC) that is the size of a standard microtiter plate. The method under development also will include reagents necessary to generate full-length, random-primed RNA-seq libraries from as little as 10 ng of total RNA.

Herein, we show general performance characteristics to date of our Fluidigm method and compare our initial results to those obtained from Illumina® TruSeq® Stranded mRNA (PN 20020594).

Methods and Materials

To demonstrate preliminary performance, we processed samples containing 4 replicates each of 10 ng and 100 ng from Universal Human Reference RNA (UHRR, Agilent® PN 740000) and human brain RNA (BioChain PN R1234035) spiked with ERCC RNA Spike-In Mix 1 and Mix 2 respectively (Thermo Fisher Scientific PN 4456740). The 32 libraries (16 generated from Fluidigm reagents and 16 from Illumina reagents) were sequenced together on an Illumina HiSeq® 2500 system using PE75 bp reads to an average sequencing depth of 102.8 million reads per sample.

FASTQ files were processed with STAR to generate alignment files and with kallisto for gene and transcript quantification. Data was aligned to NCBI RefSeq GRCh38. Sequencing metrics and figures were generated using BEDTools, SAMtools, Picard, Python®/pandas and R/ggplot2.

Conclusions

Results herein demonstrate:

- Input range from 10 to 100 ng of total RNA.
- Solid-phase capture of poly(A) RNA
- <6% ribosomal RNA reads
- >99% replicate correlation
- High-concordance of both gene-level and fold-change detection among samples processed with either Fluidigm or Illumina reagents
- Comparable dynamic range and linearity of response using ERCC spike-in controls

In conclusion, we have shown initial results from an RNA-seq method that yields comparable performance to a commonly used method using an automated platform that substantially minimizes manual pipetting steps and hands-on time. The nanoscale microfluidics-based technology enables significantly reduced reagent consumption, which can be helpful for laboratories in managing costs.

Results

The Juno system automates RNA sequencing library preparation for subsequent NGS analysis

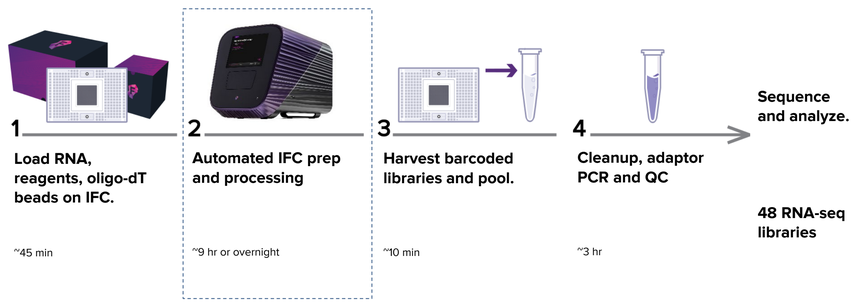
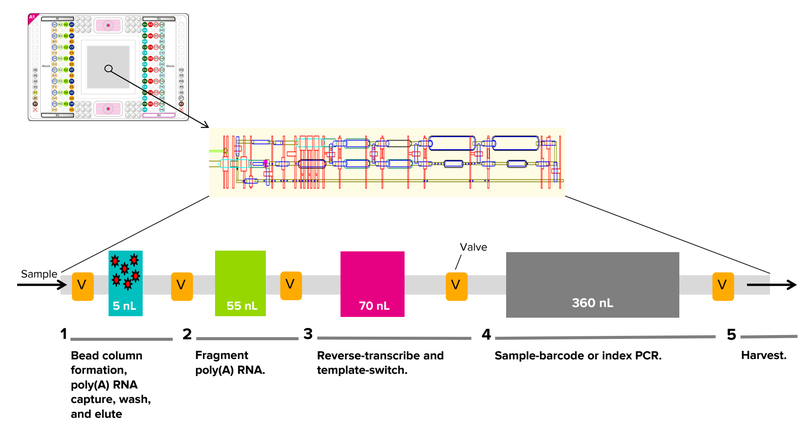


Figure 1. Samples and reagents are loaded on an IFC that automates many tedious, hands-on reaction steps to generate up to 48 RNA-seq libraries. The nanoscale microfluidic design of the IFC significantly reduces reagent consumption, which helps minimize overall analysis costs per sample.

Developing a novel IFC format for solid-phase capture and multistep, nanoliter-scale reactions



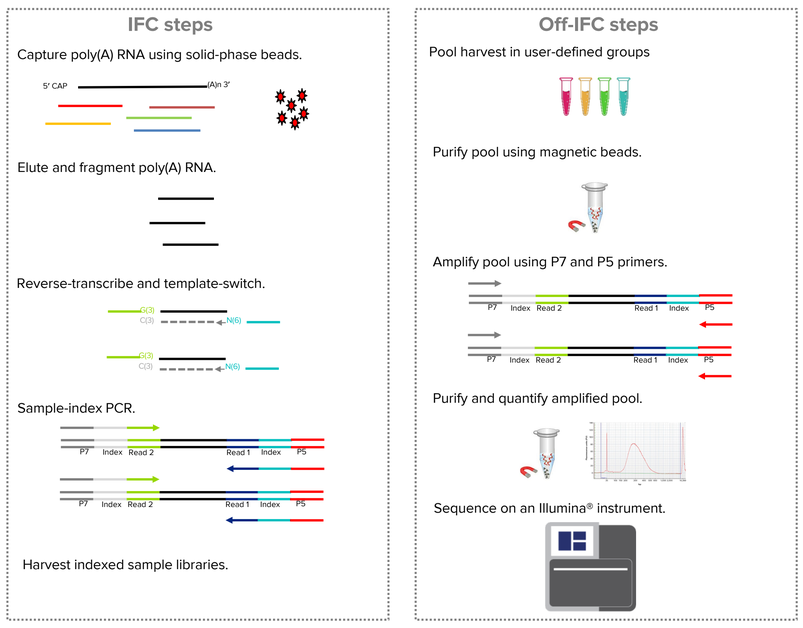


Figure 2. Total RNA samples and sample barcode indexes are loaded into their corresponding inlets along with common reagents on the IFC. Solid-phase capture, washing and elution of the poly(A) RNA occurs followed by fragmentation and a random primed reverse transcription to generate full-length libraries. Template switching generates primer sites on the end of the cDNA for sample index PCR on the IFC. Finally, libraries are harvested and pooled in user-defined groups followed by purification and quantification prior to sequencing.

Metric	Reagents: Fluidigm	Reagents: Illumina
Percent ribosomal RNA (rRNA) reads per sample	<6%	<6%
Percent genomic mapped reads per sample (not including mtRNA and rRNA)	>80%	>80%
Percent unmapped reads per sample	<3%	<3%
Technical replicate Pearson's correlation (R) within input amounts	>99%	>99%
Technical replicate Pearson's correlation (R) between input amounts (i.e., 10 vs 100 ng)	>95%	>95%

Table 1. Initial performance characteristics of samples processed with Fluidigm reagents and Illumina reagents.

Comparing gene detection per sample for libraries generated with Fluidigm and Illumina reagents

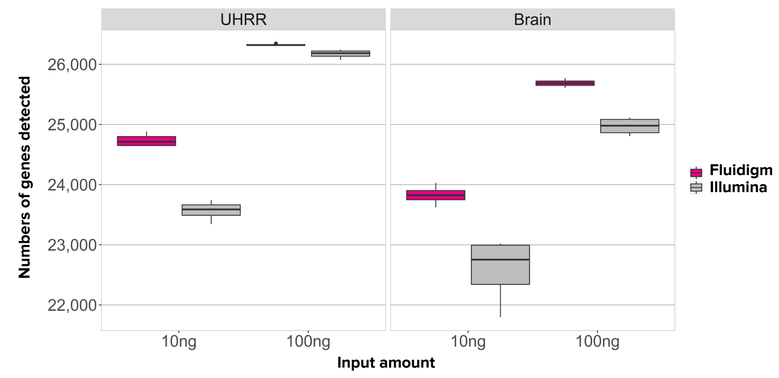


Figure 3. Gene detection from UHRR and brain were consistently observed to be higher from samples prepared with Fluidigm reagents than with samples prepared with Illumina reagents from both 10 ng and 100 ng starting sample inputs. FASTQ files were down-sampled to 30M reads per sample to equalize read depth.

Gene detection highly concordant among samples processed with either Fluidigm or Illumina reagents

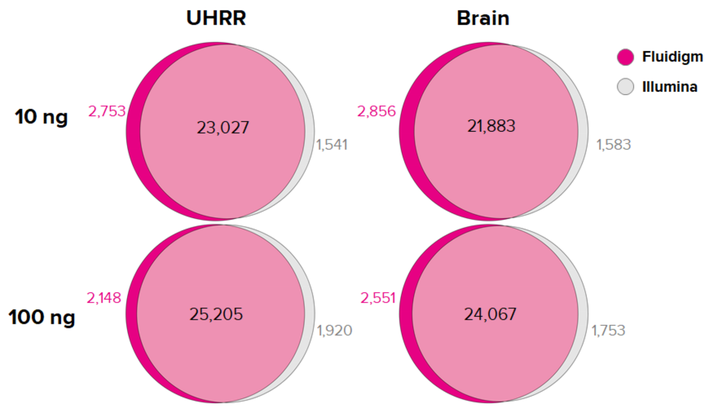


Figure 4. Gene-level detection of samples processed with either Fluidigm or Illumina reagents were observed to be in agreement in both 10 ng and 100 ng from UHRR and brain samples.

Differentially expressed gene (DEG) detection highly concordant among samples processed with both Fluidigm and Illumina reagents

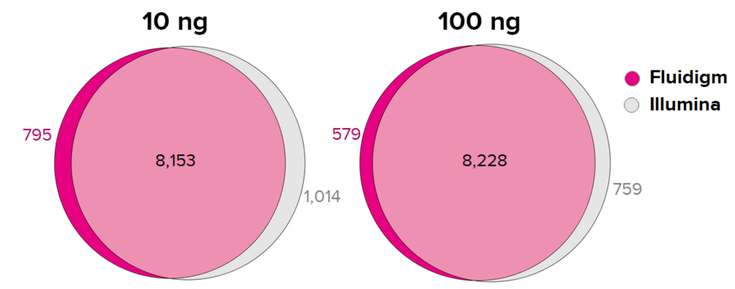


Figure 5. DEG are in agreement in both 10 ng and 100 ng from UHRR and brain samples regardless of reagents used to process samples. FASTQ files were down-sampled to 30M reads per sample to equalize read depth.

Dynamic range and linearity of response

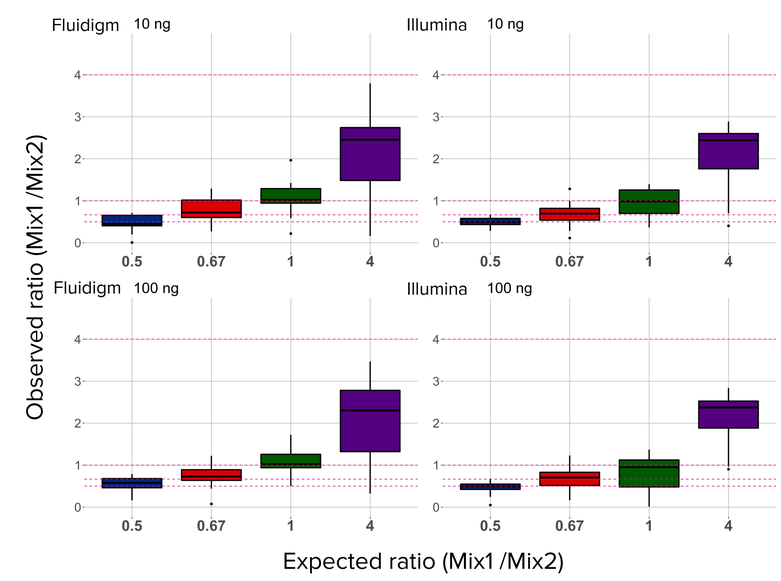


Figure 6. Comparable performance was observed for ERCC ratio relative to the expected ratio for all samples regardless of reagents used for processing. Expression values were normalized using the ERCCs of 1:1 ratio. The ratios of normalized expression values were then obtained by dividing ERCC Mix 1 samples by ERCC Mix 2 samples.

*The reagents and methods discussed herein are currently in development and subject to change without notice.

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