Expression Profiling of T Cells Using Nanoscale Automation with a Full-Length RNA Sequencing Kit on a Microfluidic Circuit Platform

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Background

Fluidigm Corporation has developed a next-generation RNA sequencing kit that combines the 48.Atlas IFC format for solid-phase poly(A) RNA capture and multistep reactions enabling processing of up to 384 samples with a single click on our instrument.

Methods and Materials

In this poster, we present a comprehensive evaluation of our new microfluidic circuit RNA-seq kit, the 48.Atlas IFC, which is one of our most versatile workflows. We sequenced libraries from as little as 10 ng of total RNA to generate high-quality RNA-seq data from multiple organs. Herein, we study comprehensive performance characteristics from three internal studies.

Results

This study demonstrates the performance of 48.Atlas IFC, with an average 85.3% percent reads mapped to transcriptome (RefSeq). Also, average gene-level Pearson's correlation at all dilution series of UHRR that included input RNA amounts below our minimum of 10 ng. To determine assay robustness, we conducted an internal analytical validation of the Juno NGS system with RNA-seq workflow leveraging the Juno™ instrument called the Advanta™ RNA-Seq NGS Library Prep Kit (PN 101-9187). Our RNA-Seq Kit supports simultaneous processing and analysis of up to 384 samples per Juno run.

Table 1. Performance characteristics of the 48.Atlas IFC RNA-Seq on 3 lots of UHRR that included input RNA amounts below our minimum of 10 ng. Performance characteristics (technical replicates within input amounts) of the Juno NGS system enable an automated, cost-effective approach to RNA sequencing.

<table>
<thead>
<tr>
<th>RNA Source</th>
<th>Total Read Percent</th>
<th>Percent Reads Mapped</th>
<th>Percent Reads Mapped Non-mitochondria</th>
<th>Percent Reads Mapped Non-rRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>UHRR</td>
<td>86.8%</td>
<td>98.1%</td>
<td>86.8%</td>
<td>86.8%</td>
</tr>
<tr>
<td>Brain</td>
<td>77.0%</td>
<td>98.0%</td>
<td>77.0%</td>
<td>77.0%</td>
</tr>
<tr>
<td>Thyroid</td>
<td>67.0%</td>
<td>98.0%</td>
<td>67.0%</td>
<td>67.0%</td>
</tr>
</tbody>
</table>

Discussion

Herein, we present performance characteristics of the 48.Atlas IFC RNA-Seq workflow on a Microfluidic Circuit Platform. The Juno NGS system automates the RNA-seq workflow along with a new, total paired-end 75 bp reads. RNA samples were Universal Human Reference RNA (UHRR, Agilent® PN 740000) and human brain RNA (BioChain® PN R1234035). Input samples were processed on the Juno instrument. The system solution automates many tedious hands-on steps to processed RNA samples, which helps minimize overall costs per sample.

Conclusion

Results herein have shown excellent mapping rates of ≥85% with low percent rRNA reads ≤2.2% and Pearson's correlation coefficients ≥0.984 at all conditions. Gene expression was quantified with high reproducibility with technical replicates within input amounts (10 ng). The 48.Atlas IFC NGS kit enables excellent high-throughput generation of RNA-seq data, significantly minimizing hands-on time and costly reagent consumption, which helps to facilitate the incorporation of RNA-seq workflows into the immunology research workflow.