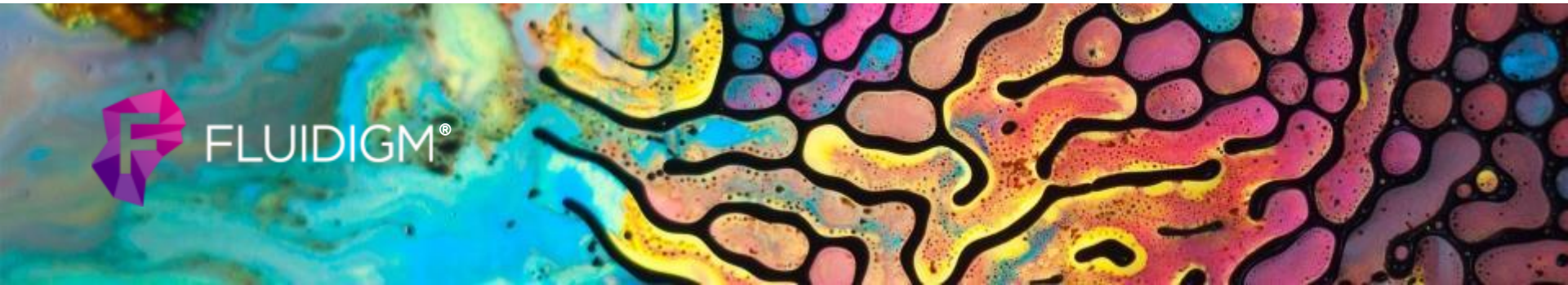
C1 Total RNA-Seq

Analysis of polyadenylated and non-polyadenylated RNA to fully characterize the single-cell transcriptome in mammalian cells

Single-Cell Genomics Team

September 18, 2018



Study objective

- Four cell types were tested to examine the performance of the C1 Single-Cell Stranded Total RNA application (C1 Total RNA-Seq).
- To evaluate the efficiency of Total RNA-Seq in comparison to a poly(A)-based method, side-by-side experiments were performed using the SMART-Seq® v4 Ultra Low Input RNA Kit.

	K562 cells	HL60 cells	Stimulated T cells	HeLa cells
Total RNA-Seq (TR)	2 IFCs*	2 IFCs*	2 IFCs	2 IFCs
SMART-Seq v4 (SSv4)	2 IFCs	2 IFCs	2 IFCs	1 IFC

*Initial TR experiments with K562 and HL60 cells were run with the SMARTer Stranded Total RNA-Seq Kit v2 – Pico Input Mammalian. The remaining TR experiments were performed using the updated SMART-Seq® Stranded Kit after its launch in May 2018.

Assessing performance

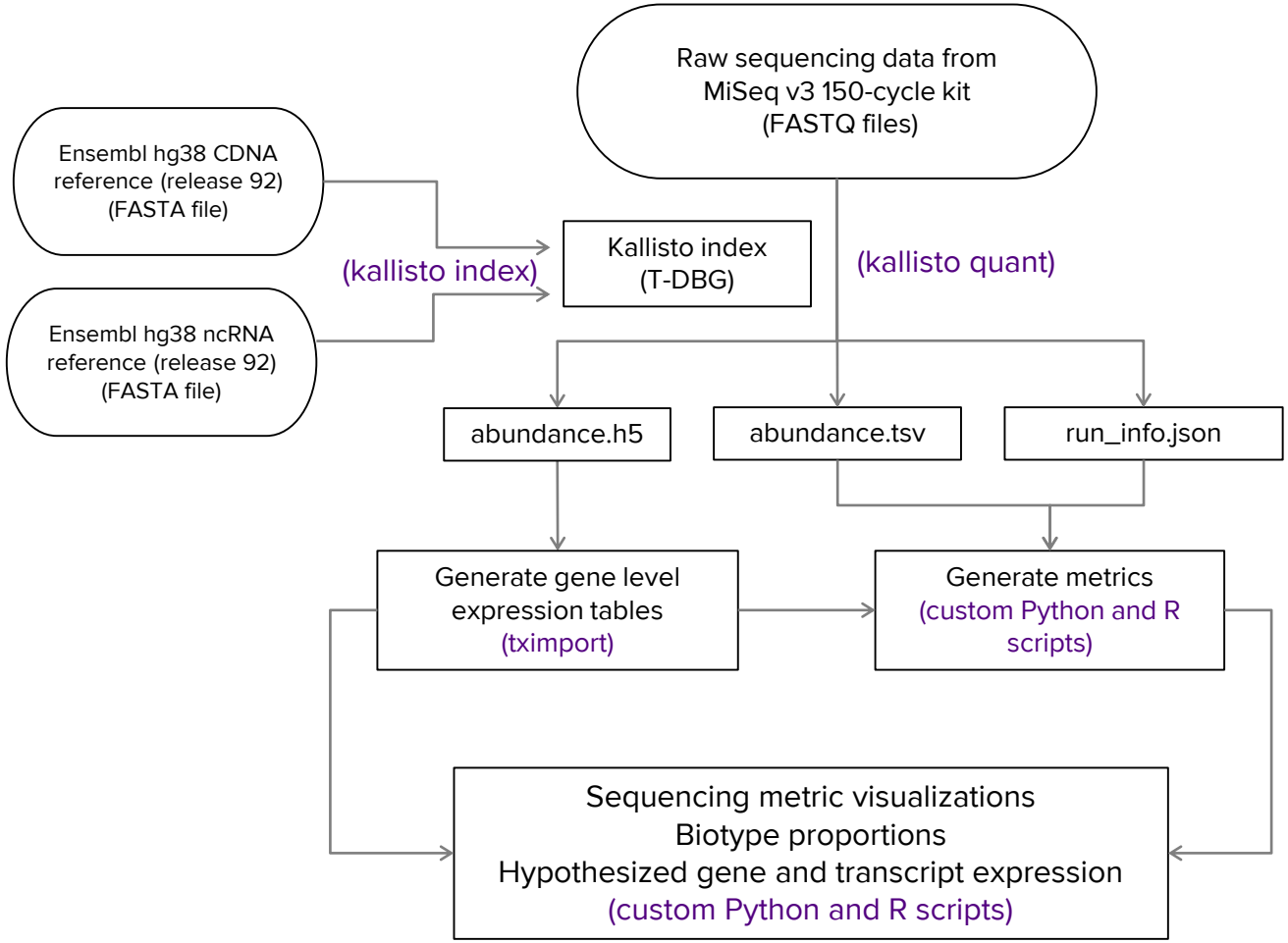
1. Quality control metrics for sample selection.

2. Comparison of sequencing metrics between C1 Total RNA-Seq and C1 SMART-Seq v4.

3. Evaluation of ncRNA detection.

4. Assessment of non-poly(A) detection.

Bioinformatic analysis workflow

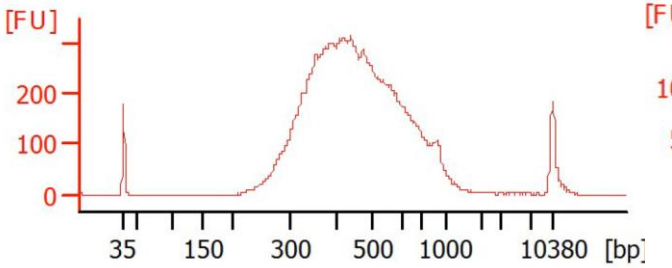


FASTQ files were also mapped to visualize transcript coverage (bowtie, picard)

Total RNA-Seq library profiles

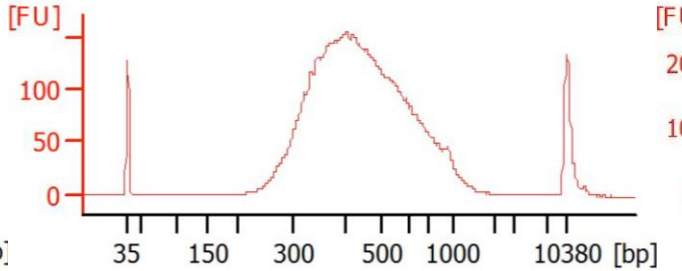
Agilent Bioanalyzer High Sensitivity Kit

K562 cells^



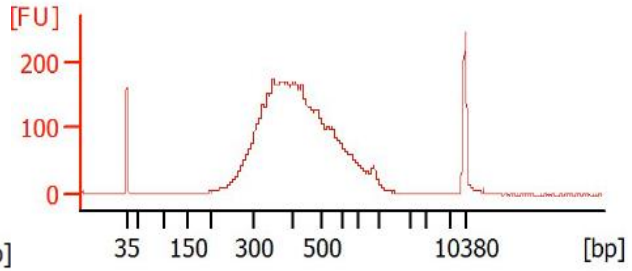
Pool of 96 K562 cells

Stimulated T cells^



Pool of 48 stimulated T cells

HeLa cells^^



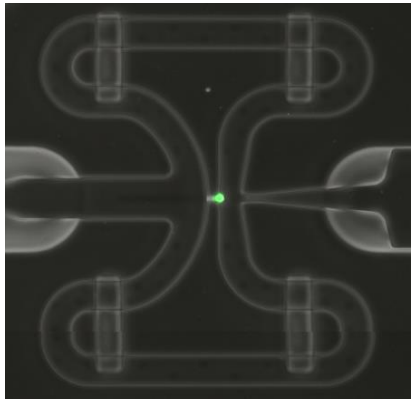
Pool of 48 HeLa cells

^Libraries for K562 cells and stimulated T cells went through 13 cycles of amplification during PCR2 and were diluted 1:10 prior to loading on the Bioanalyzer.

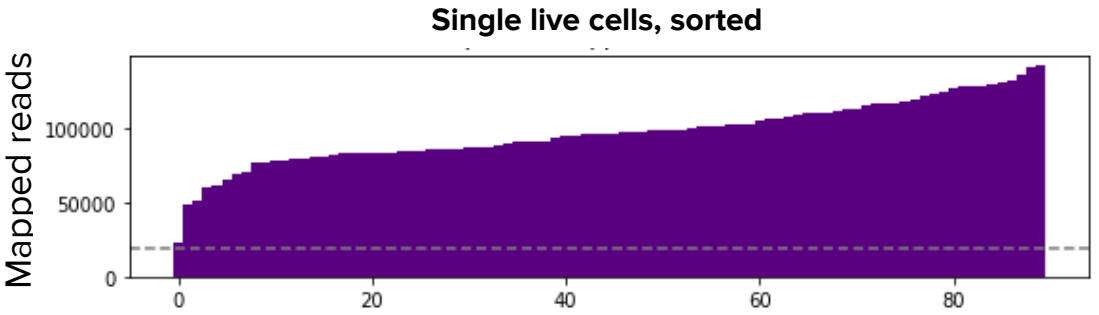
^^Library for HeLa cells went through 12 cycles of amplification during PCR2 and no dilution was made prior to loading on the Bioanalyzer.

Processing live cells

Only cells with >20,000 mapped reads received secondary analysis



Single live cell

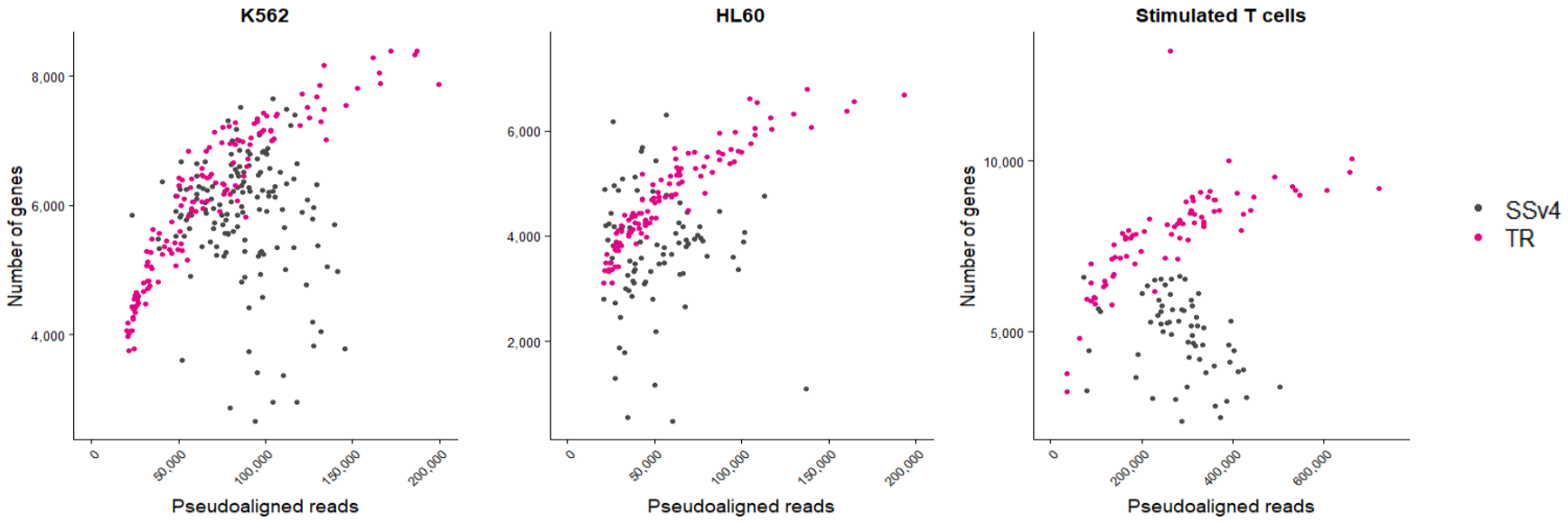


One IFC shown as an example. Dashed line indicates 20,000 mapped reads.

	K562	HL60	Stimulated T cells*
Smart-Seq v4 (SSv4)	57	40	38
	90	42	24
Total RNA-Seq (TR)	58	69	38
	83	45	40

Number of cells passing filters to receive secondary analysis for each IFC.
*Stimulated T cells were analyzed in pools of 48 cells.

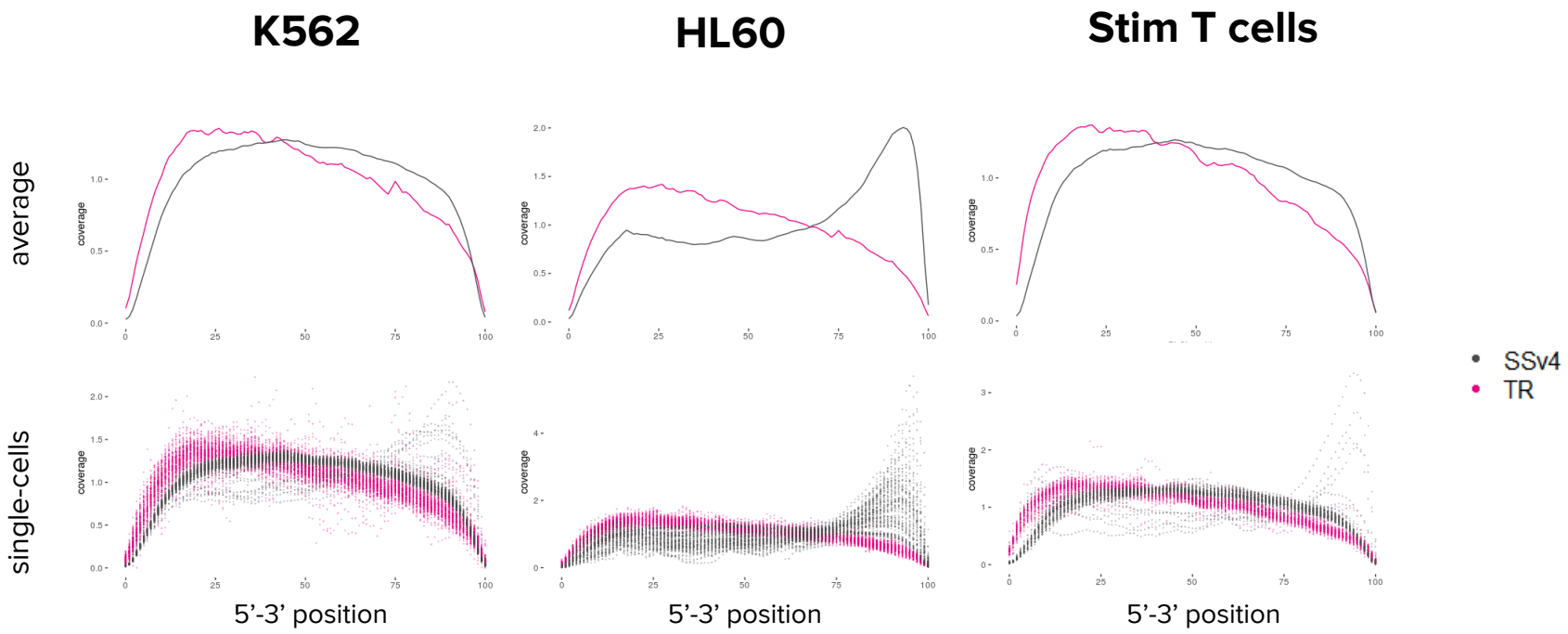
Total RNA-Seq shows higher gene detection with increasing mapped reads



Gene counted if TPM > 1

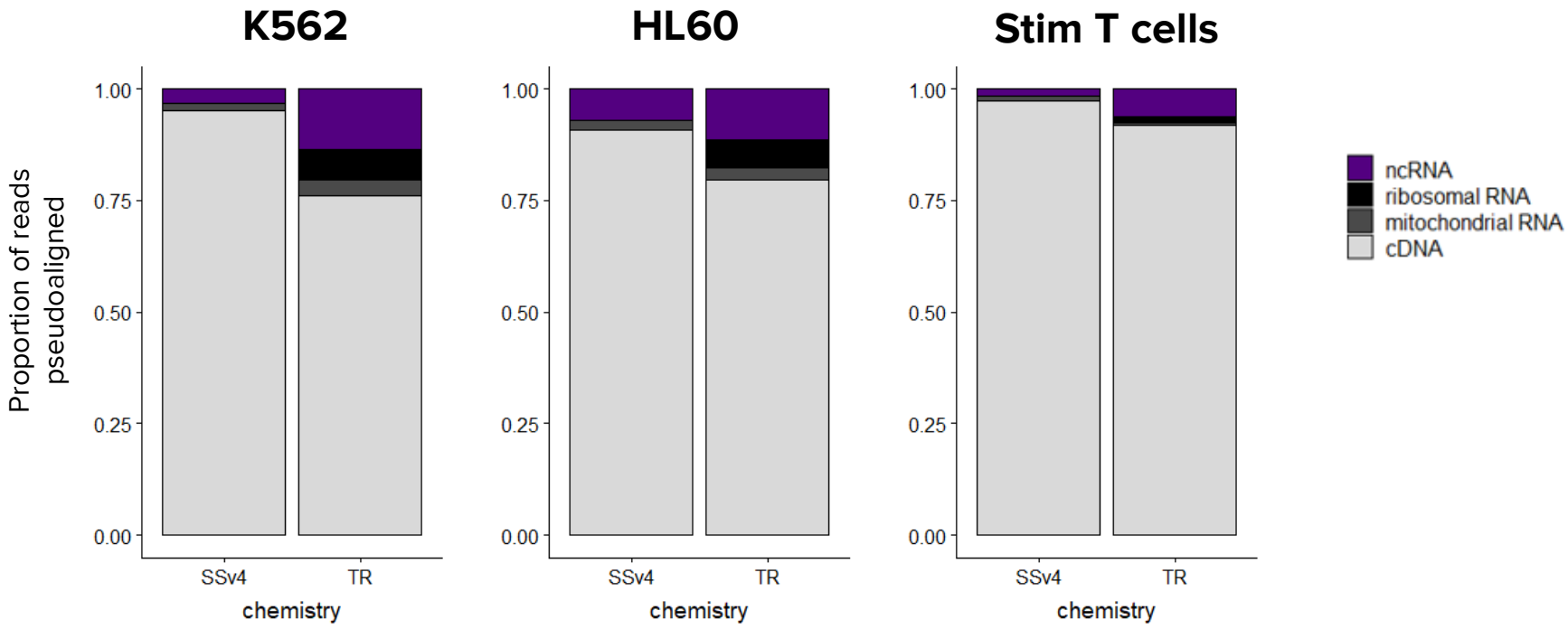
Total RNA-Seq demonstrates a deeper cell characterization methodology providing novel data on non-poly(A) RNA features aiding full single-cell transcriptome analysis.

Total RNA-Seq shows similar or better transcript coverage than SMART-seq v4



In HL60 cells where SMART-Seq v4 shows a strong 3′ bias, total RNA-Seq maintains full-length coverage with little 5′–3′ bias.

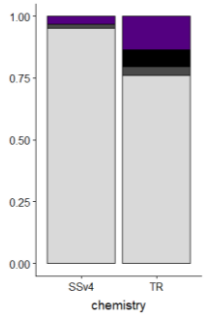
Total RNA-Seq exhibits a greater proportion of reads to noncoding RNA



Gene level biotypes and counts used

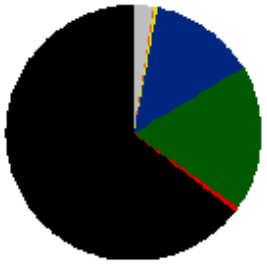
Total RNA-Seq demonstrates a deeper cell characterization methodology providing novel data on non-coding RNA features aiding full single-cell transcriptome analysis.

Total RNA-Seq captures a greater diversity of non-coding RNA compared to SSv4

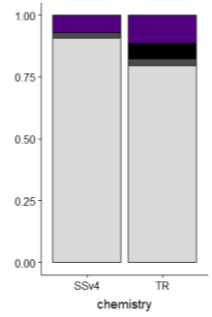


K562

SSv4 TR

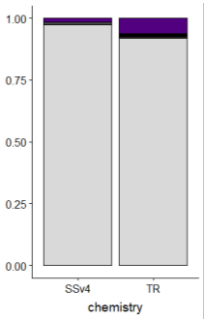


% of reads to ncRNA 3.7 13.6



HL60

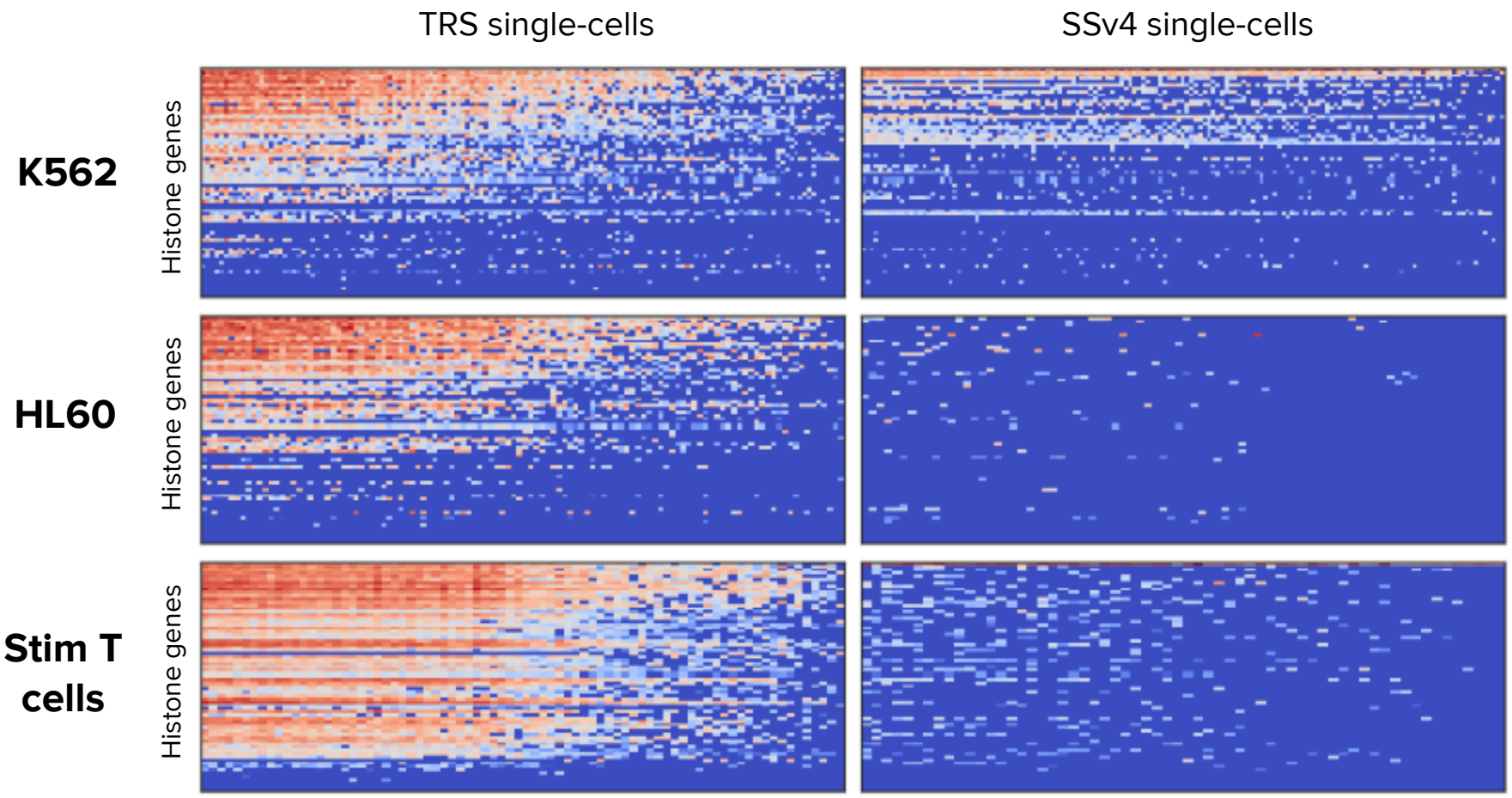
% of reads to ncRNA 6.9 11.4



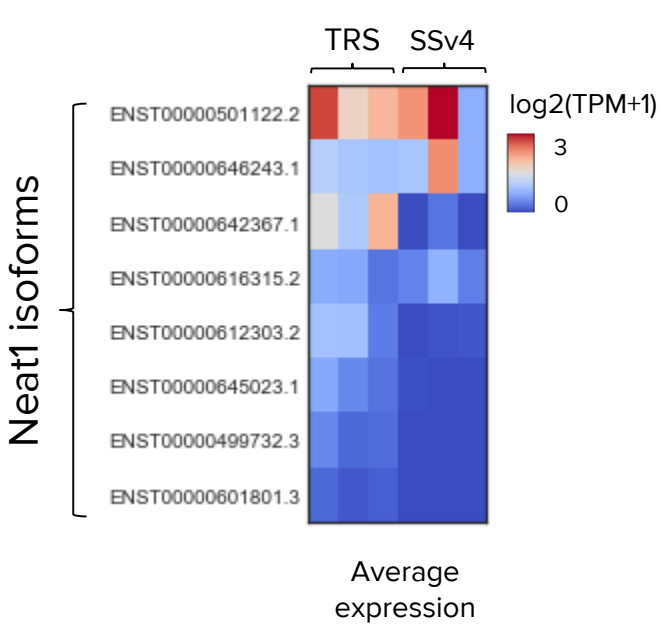
Stim T cells

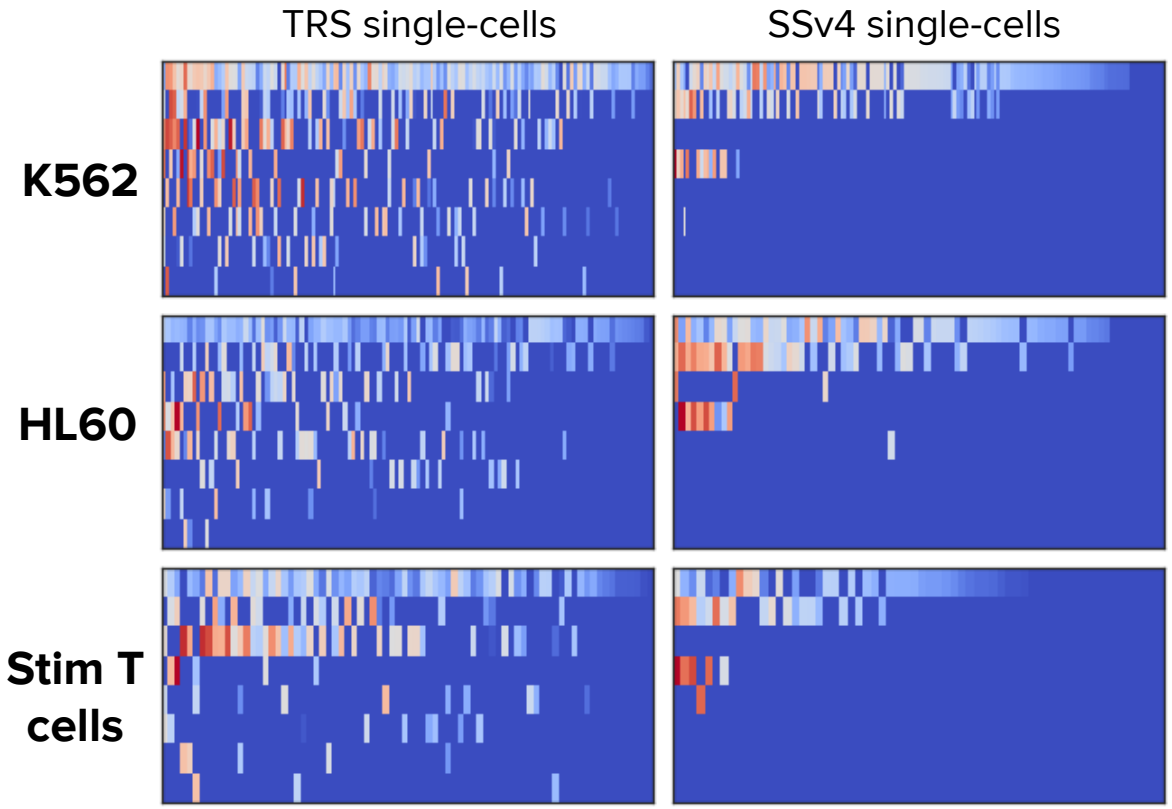
% of reads to ncRNA 1.5 6.2

Total RNA-seq detects more non-poly(A) histone genes than SSv4



Total RNA-Seq detects more non-poly(A), lncRNA Neat1 isoforms than SSv4





Total RNA-Seq
Summary

- Demonstrates a deeper cell characterization methodology providing novel data on non-poly(A) RNA features aiding full single-cell transcriptome analysis.

- Maintains full length coverage with little 5’–3’ bias in cell types where SMART-Seq v4 shows a strong 3′ bias.

- Detects more non-poly(A) transcripts than SMART-Seq v4.

- Provides a method for researchers to perform deeper single-cell characterization by enabling analysis of novel non-coding RNA features.

Thank you.

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