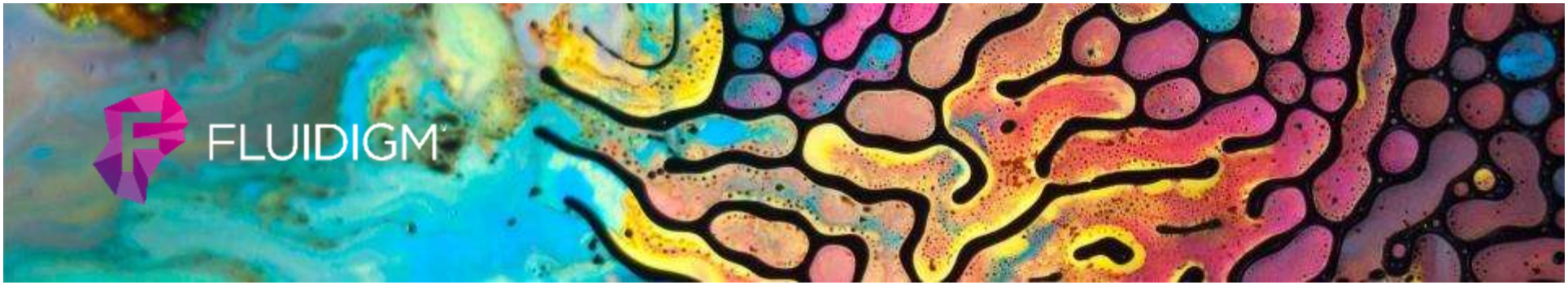
Single-Cell RNA and protein expression

Single-cell multi-omics in action

January 31, 2019



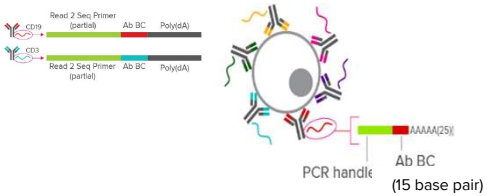
C1 REAP-seq workflow

C1 Single-Cell mRNA Seq HT IFC

1 Obtain barcoded antibodies.

2 Stain cells.

Preparation



3 Isolate antibody stained cells.

4 Image.

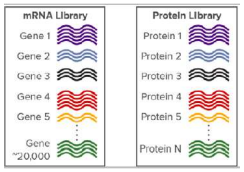
5 REAP-seq chemistry

C1™ processing

6 Library preparation.

7 Sequence and analyze.

Data collection and analysis

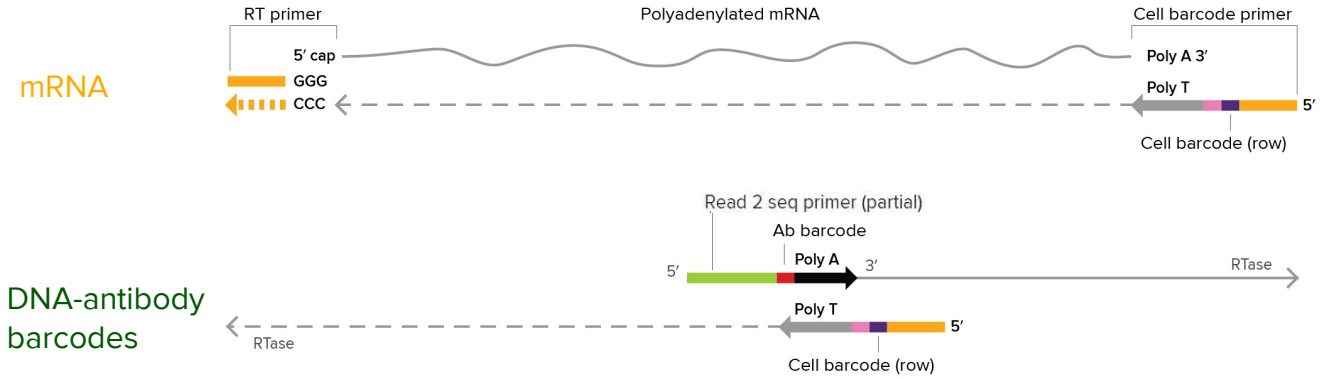


>500 bp ~193 bp

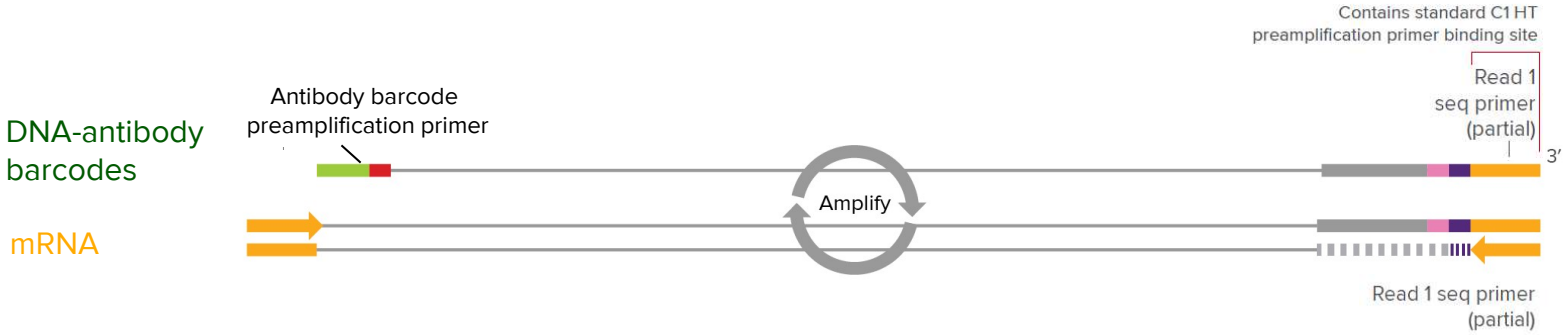
C1 REAP-seq chemistry

Single-Cell mRNA Seq HT* IFC

1. Reverse transcription



2. Preamplification



* High-throughput

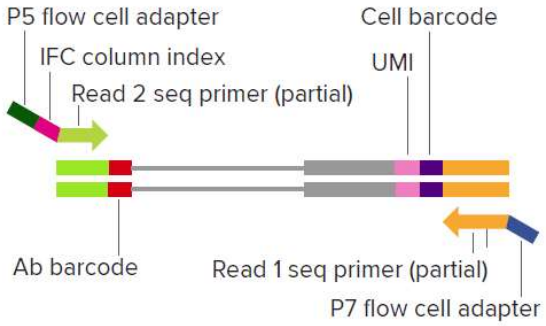
C1 REAP-seq chemistry

Single-Cell mRNA Seq HT IFC

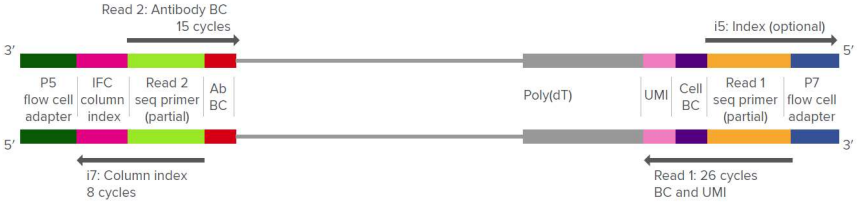
3. Harvest and cleanup to separate and process cDNA and antibody barcode library in parallel.

Antibody barcode library prep

Antibody library barcoding and adapter addition by PCR



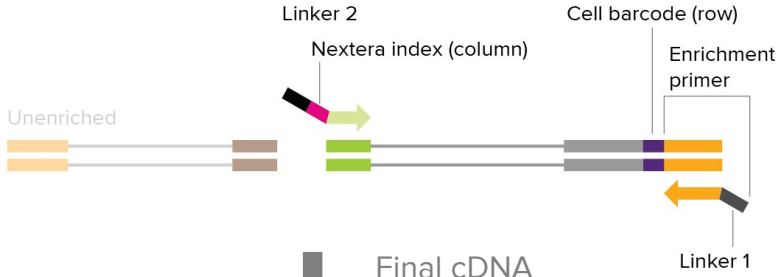
Final Ab BC library structure



cDNA library prep

Library tagmentation

3’-end enrichment



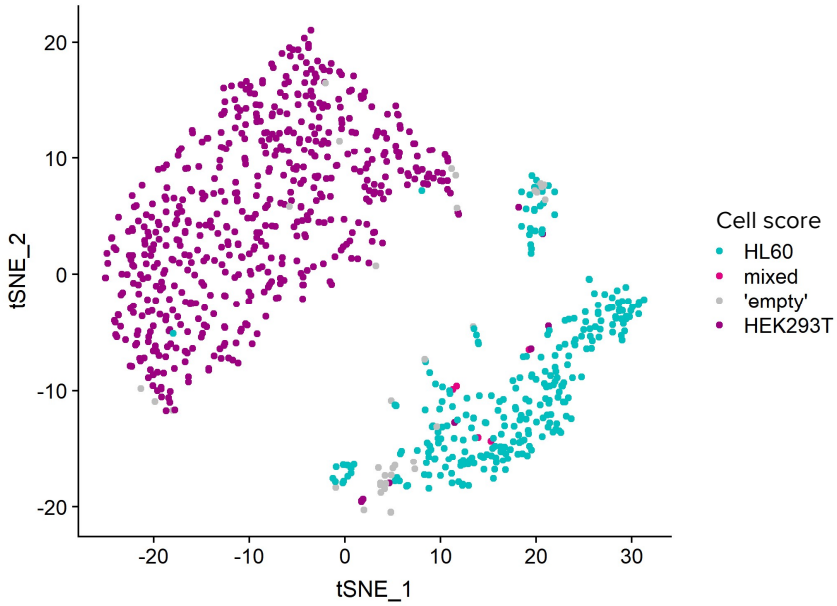
Final cDNA library structure

Methodology design

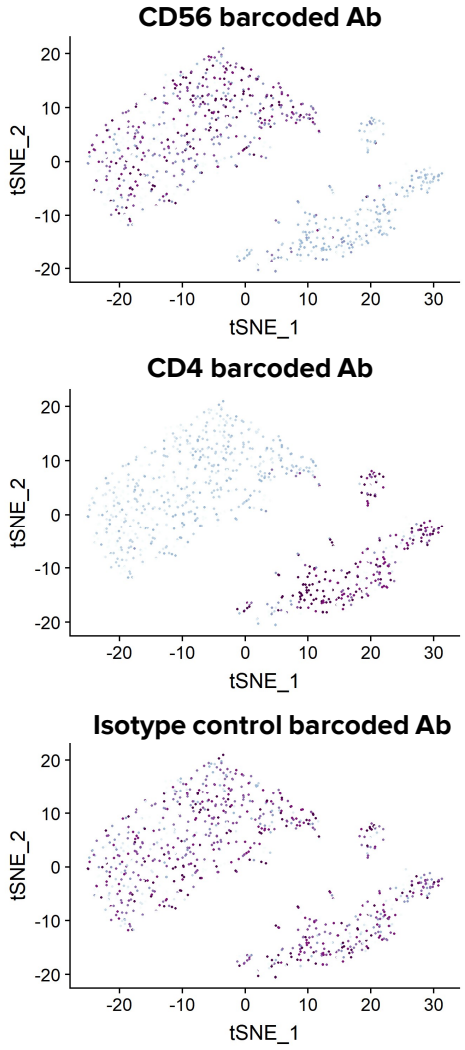
1. HEK293T cells and HL-60 cells were incubated with barcode-labeled antibodies (15 α-human, 1 α-mouse, 5 mouse/rat isotype controls).
2. The cells were then mixed 50:50 prior to loading onto 2 HT IFCs.
3. HEK293T cells and HL-60 cells expressed mCherry and GFP, respectively.
4. Imaging cells prior to processing informs downstream sequencing data.
5. Implemented a 3′ end counting chemistry for mRNA-seq and antibody barcode amplification for the antibody-barcode data. This was performed as part of the same reaction on C1 HT IFCs.
6. Cell protein and gene expression was measured by Illumina® NextSeq™.
7. Bioinformatic processing of FASTQ files was performed with Kallisto for mRNA-seq and CITE-seq Count for antibody barcode data.

Multimodal mRNA and protein analysis

Antibody barcode expression shows cell specificity



tSNE is based on mRNA-seq data



Ab BC expression is CLR normalized.

Summary

- Development data shows REAP-seq on medium HT IFC.
- Cell mixing experiments demonstrate that cell populations group as expected by mRNA expression.
- Cell-specific antibody barcodes are localized to the appropriate cell groups (CD56 expression in HEK293T cells and CD4 in HL-60 cells).

Thank you.

For Research Use Only. Not for use in diagnostic procedures.

©2019 Fluidigm Corporation. All rights reserved. Fluidigm, the Fluidigm logo and C1, are trademarks and/or registered trademarks of Fluidigm Corporation in the United States and/or other countries. All other trademarks are the sole property of the respective owners. 01/2019