C1 update

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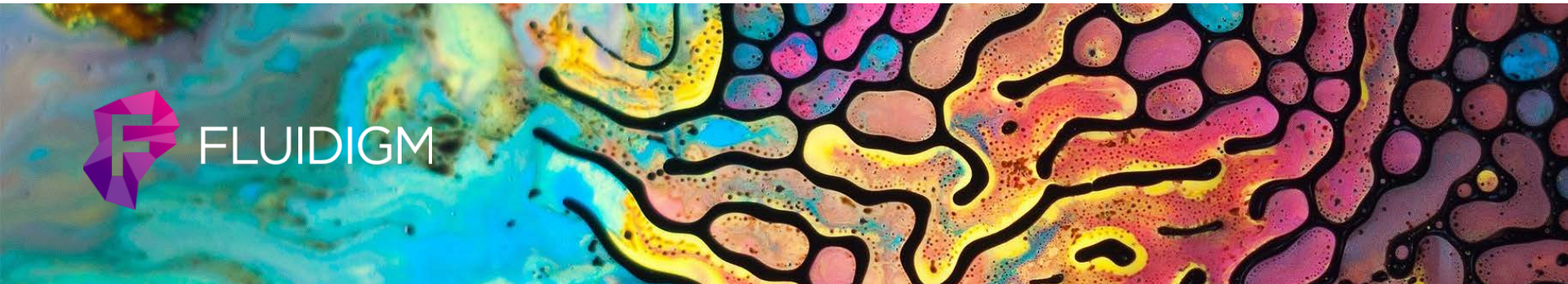
Fluidigm Corporation



Overview

Current C1 96 IFC portfolio

Introducing the HT IFC

Library prep approaches with the Fluidigm C1
3’ end counting vs. Whole transcriptome sequencing

Chemistry
Whole transcriptome – current C1 (96 IFC)
3’ end counting (HT IFC)

HT IFC Workflow

Sequencing Considerations

Competitive Update

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C1

- Single-cell isolation and preparation
- 96 and 800 cell formats
- Supports sequencing and real-time PCR

The C1 Application Portfolio

*96 cell IFC
+HT IFC

Gene expression

- mRNA Sequencing (full transcript* or 3’ end counting+)
- mRNA and miRNA targeted q-PCR*

Whole genome amplification

- Targeted Amplicon*
- Whole Exome*
- Whole Genome*

Epigenetics

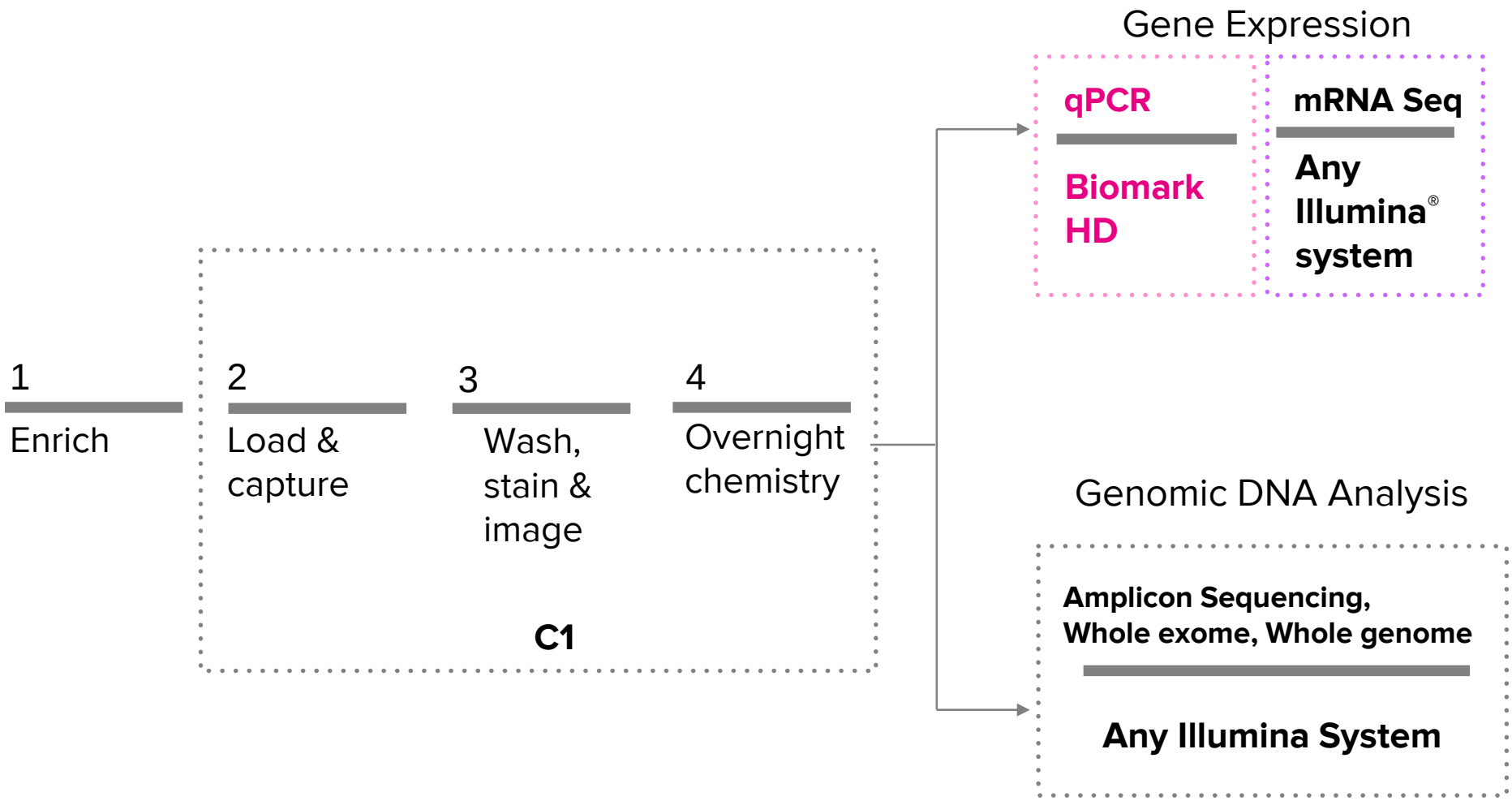
- DNA accessibility (ATAC-Seq)*
- Methylation, gene expression, and genotyping from the same cell (SC-GEM)*

Detail of mRNA seq methodologies available on the C1 system

*default method for C1 96
**default method for C1 HT

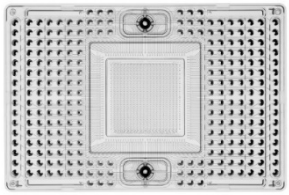
Method Name	Available on	RT Method	Sequencing method	UMIs?
STRT-Seq (Linnarsson, Karolinska, Sweden)	Open App 96 IFC	Template switch	5’ end counting	+
CEL-Seq (Yanai, Technion, Israel)	Open App 96 IFC	PolyT, T7	3’ end counting	+
CEL-Seq 2 (Yanai, Technion, Israel)	Open App 96 IFC	PolyT, T7	3’ end counting	+
SMARTer v1* (Clontech, USA)	mRNA seq 96 IFC Open App 96 IFC	Template switch	Full length	-
SMARTer v4 (Clontech, USA)	mRNA Seq 96 IFC Open App 96 IFC	Template switch	Full length	-
Smart Seq 2 (Sandberg, Karolinska, Sweden)	Open App 96 IFC	Template switch	Full length	-
CAGE-Seq (Riken, Japan)	Open App 96 IFC	Template switch	5’ end counting	+
HT IFC** (Fluidigm)	HT IFC	Template switch	3’ end counting	-

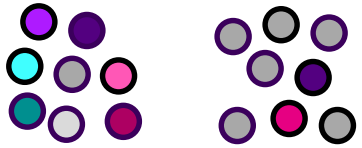
Single-cell analysis on C1



Experimental Design with C1 IFCs

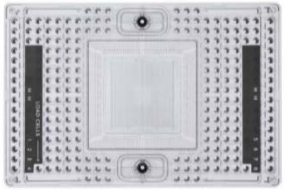
C1 HT IFC

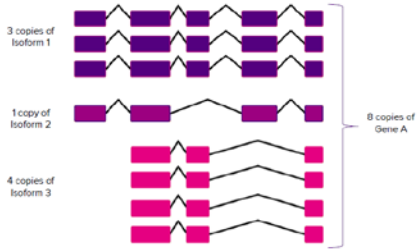




- identify subtypes/gene signatures
- sample rare cell types

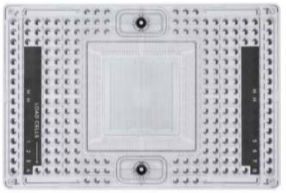
C1 96 mRNA Seq

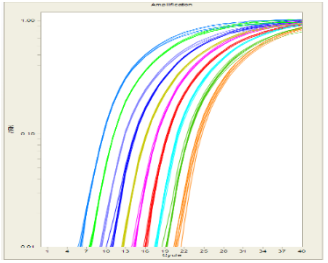




Deeper sequencing on fewer cells (splice variants, allele-specific expression, rare transcripts)

C1 96 STA





Targeted panel for validation and follow up

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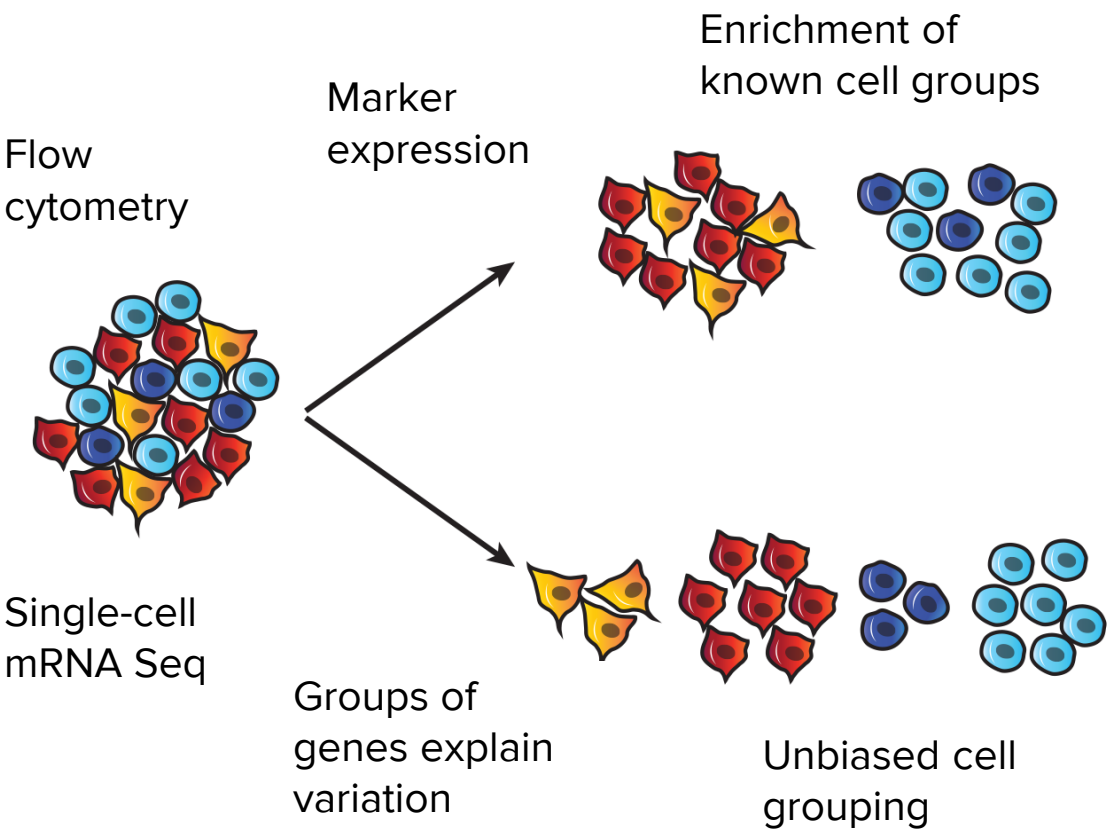
Competitive Update

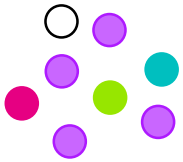
A new way of finding cell types

Pollen, A. et al, *Nature biotechnology*, 2014



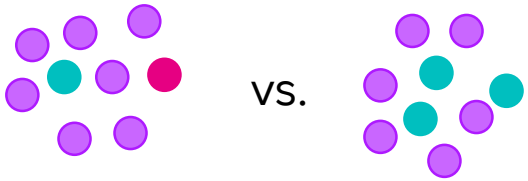
Scientific discovery in single-cell biology

How many cell types are there in my population?

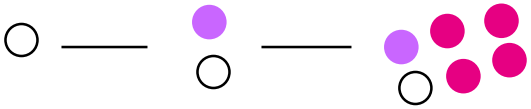


What are the rare cells and are they important?
What is each cell type doing in the larger picture?

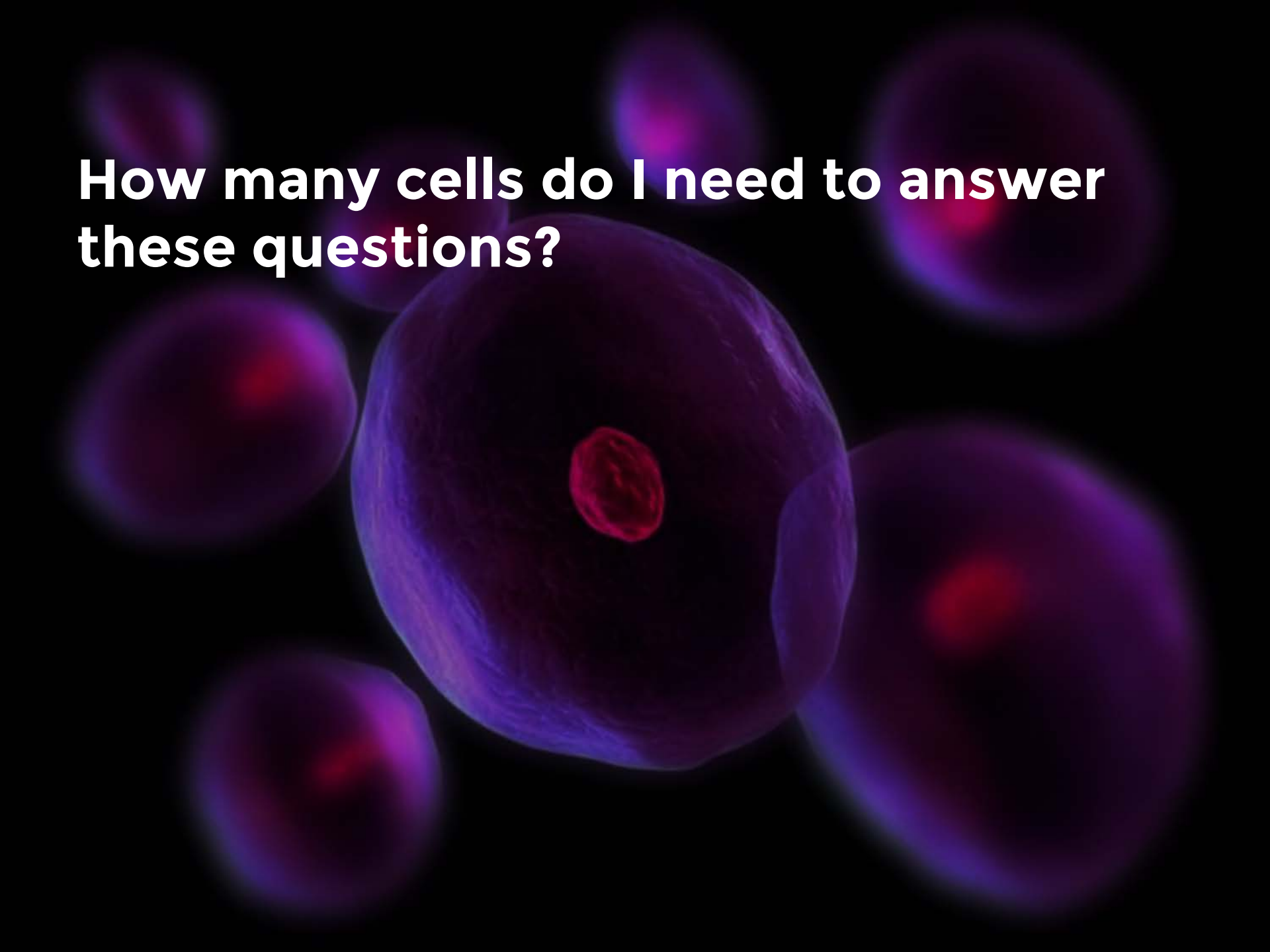
How do subtypes change between states?



How did each cell type develop?



How many cells do I need to answer these questions?

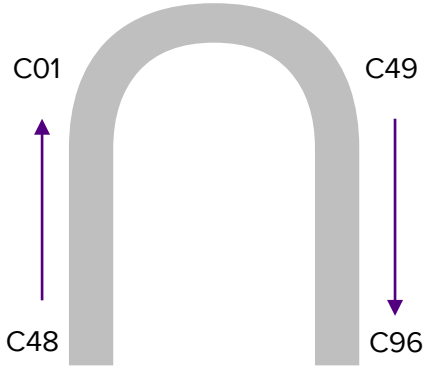


How many cells and how many reads?

	PROS	CONS
More cells, low depth/cell	• More likely to see rare cells • Overcome noisy gene expression between cells • Can be sufficient for cell type classification	• Can miss rare transcripts
Fewer cells, high depth/cell	• Rare transcripts detected • More detailed sequence-level information (splice variants, alleles)	• Rare cells can be missed • More reads required per cell for significance ($)

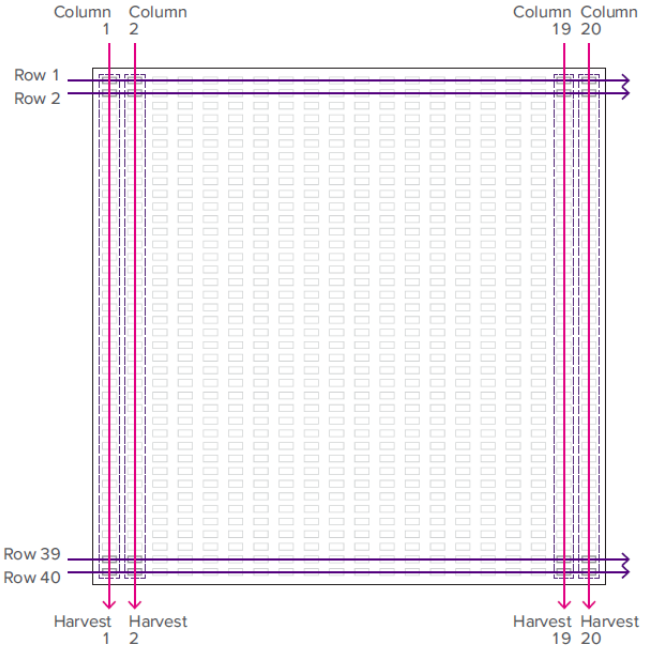
C1 96 cell IFC

96 capture sites per chip
Single sample loading inlet



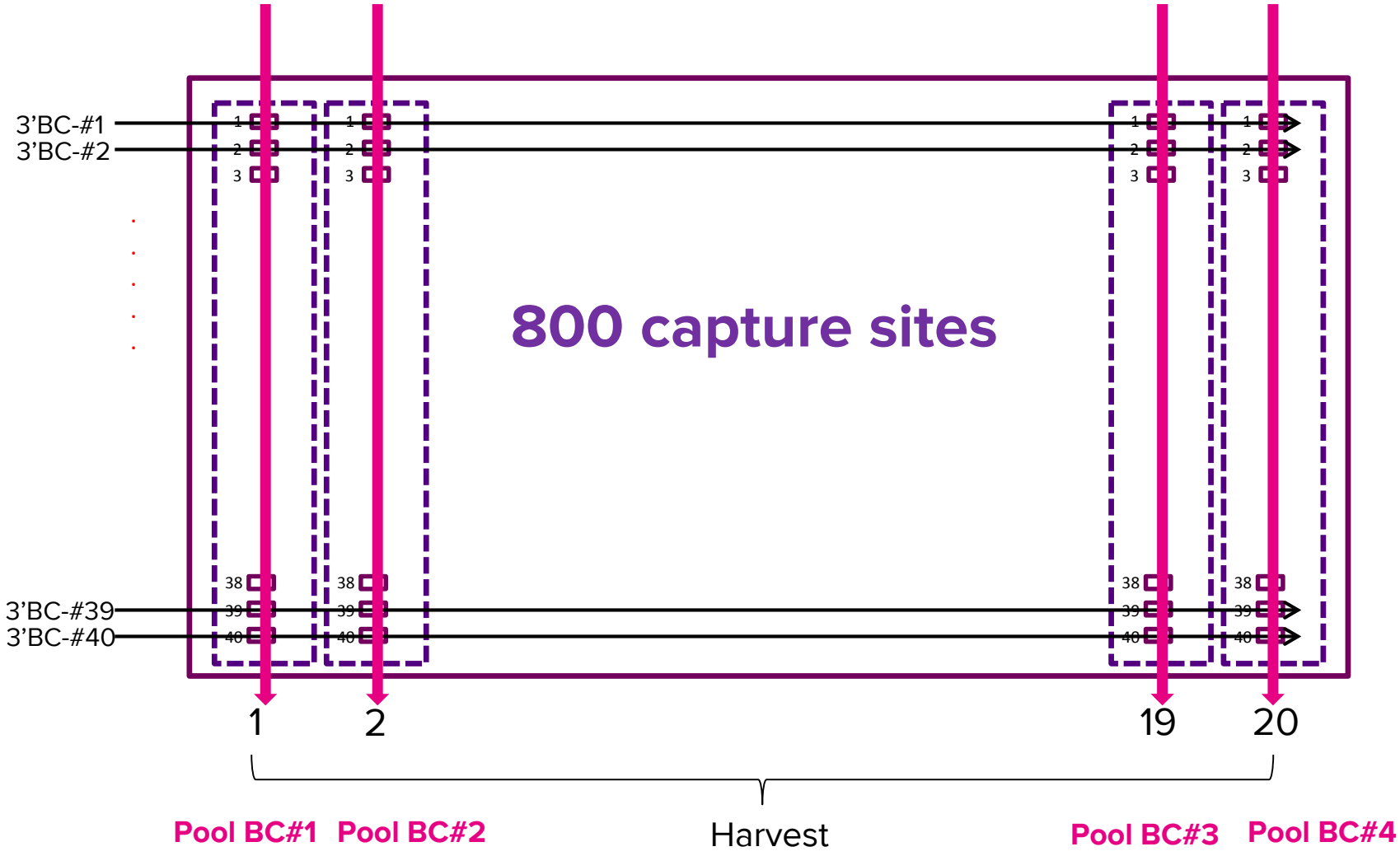
C1 HT IFC

800 capture sites per chip
2 sample loading inlets
(3’ end barcodes applied in Row orientation)

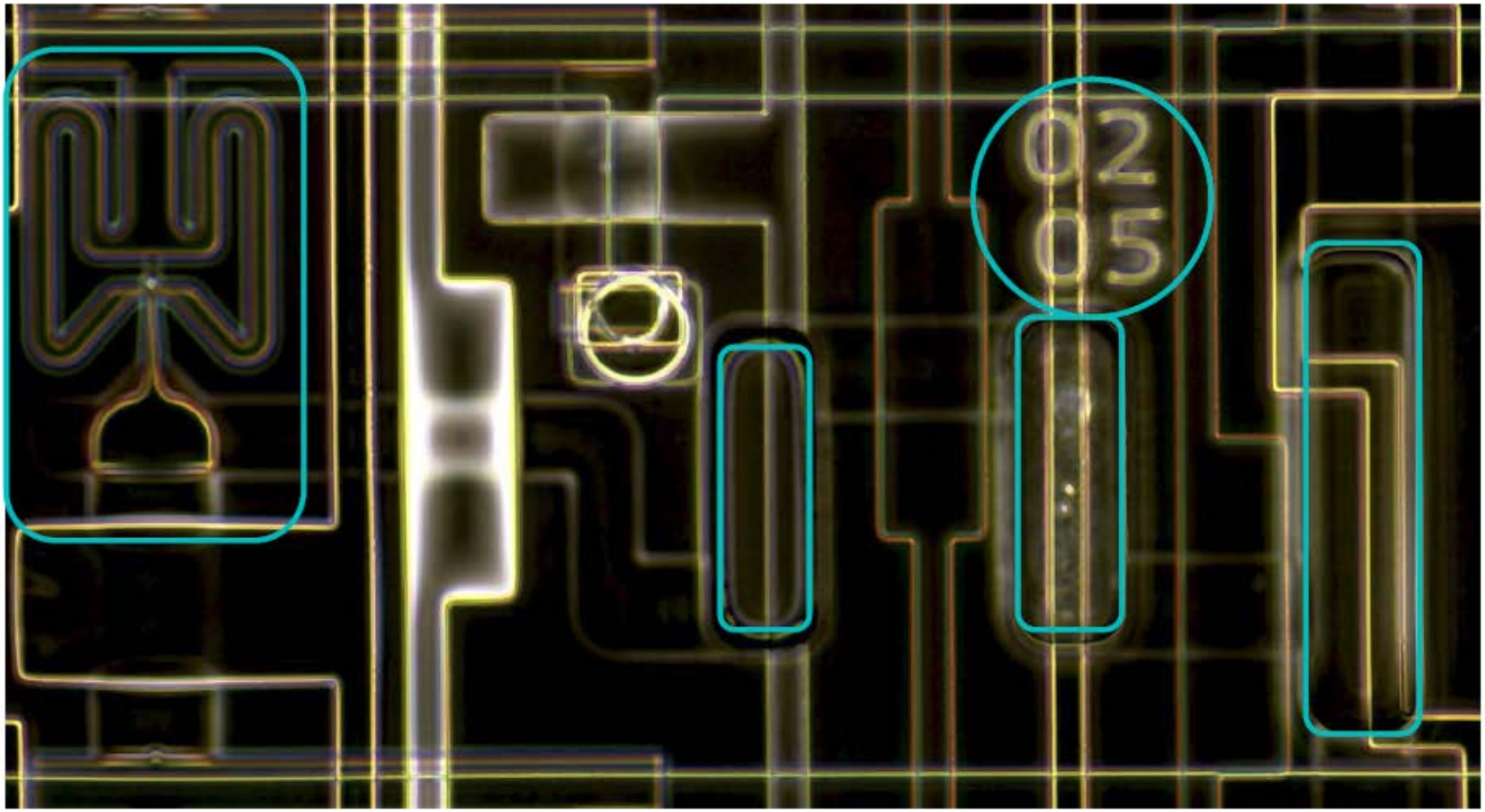


Barcoding on IFC

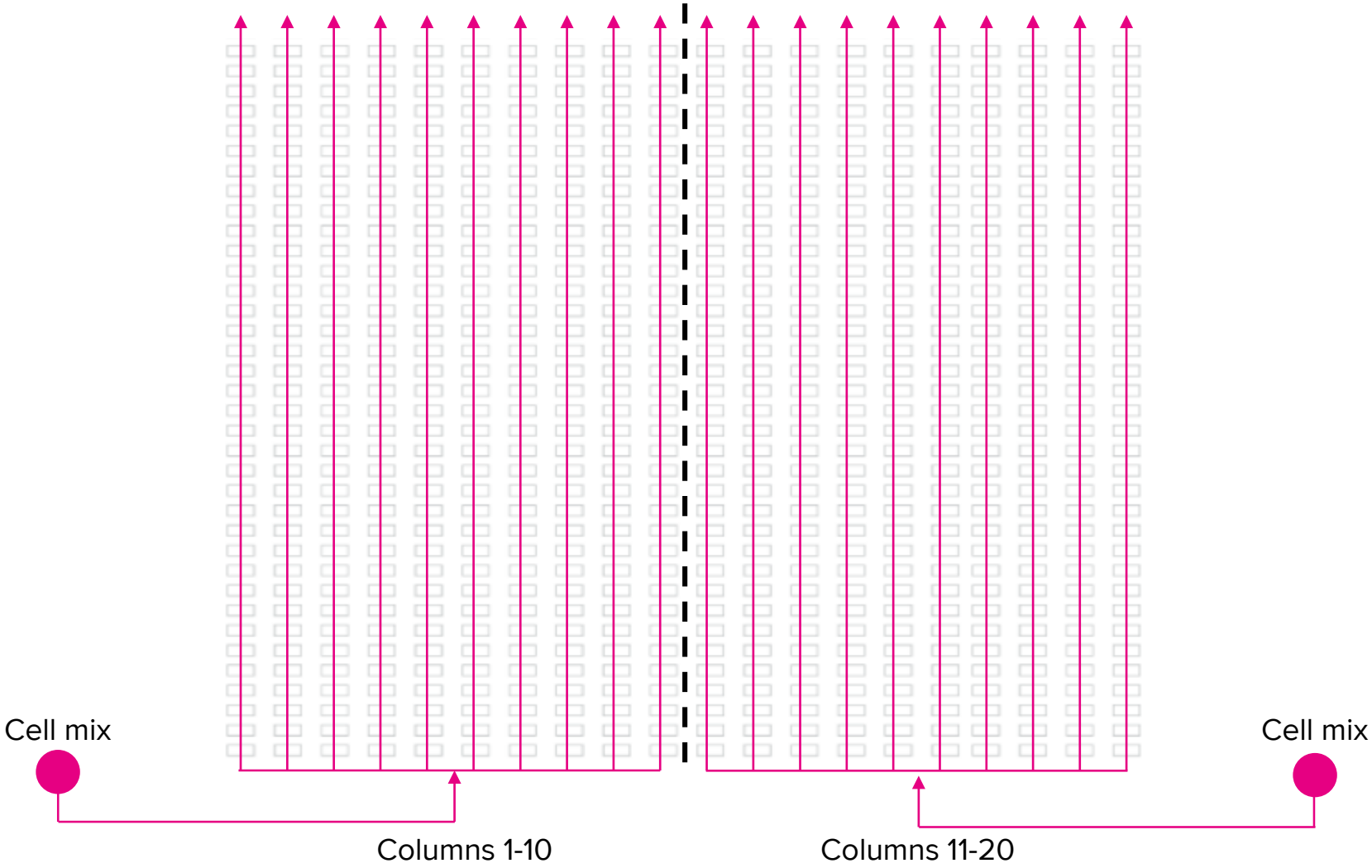
*pool BCs added in Library prep, off chip



Site locations show column and row number



Cells are loaded into capture sites in the column dimension



Area of imaging on HT IFC

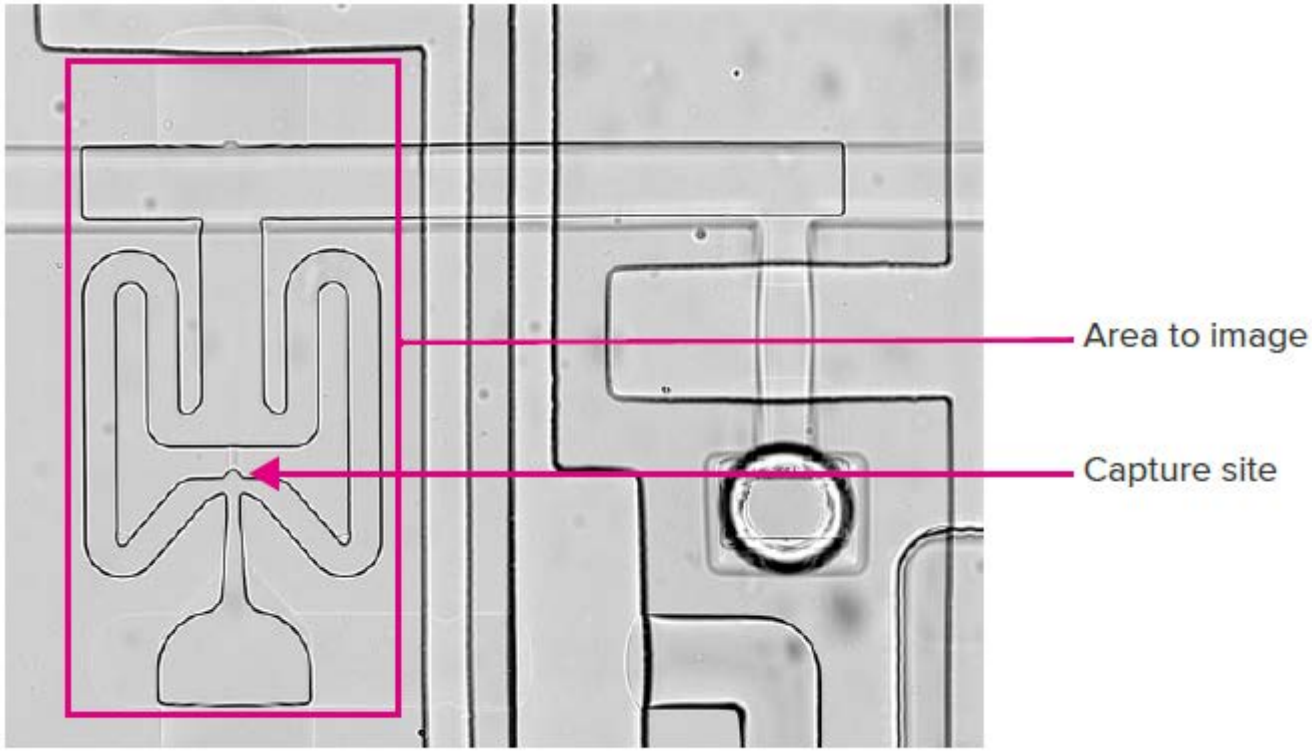
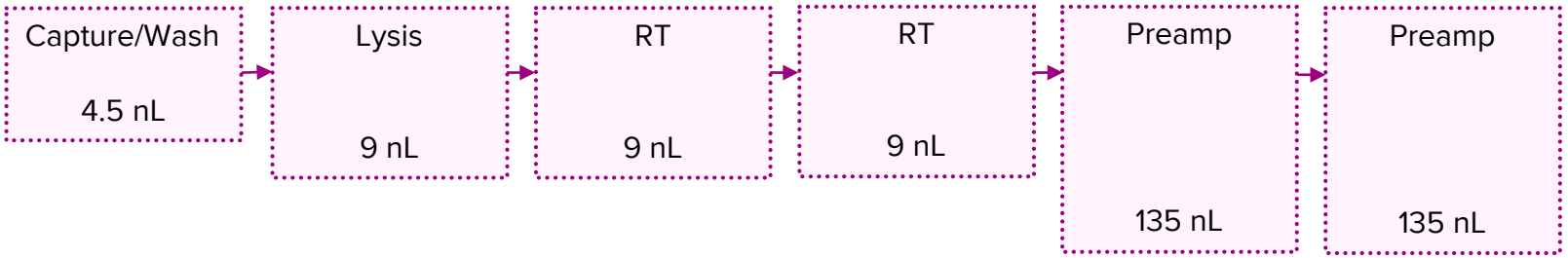


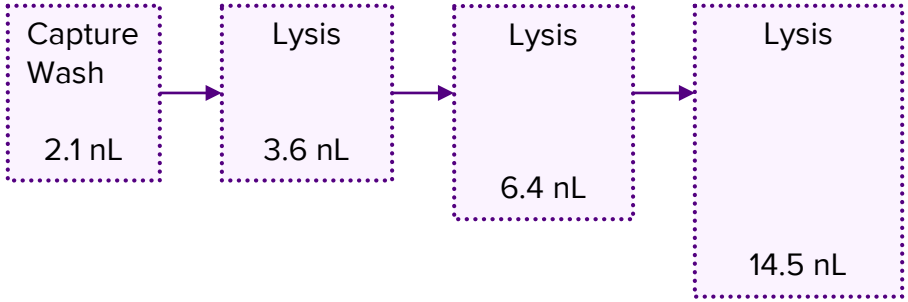
Figure 6. HT IFC cell capture area

Reaction chambers are fewer and smaller on the HT IFC

96 Cell IFC (total volume 301.5 µL)



HT IFC (total volume 26.6 µL)



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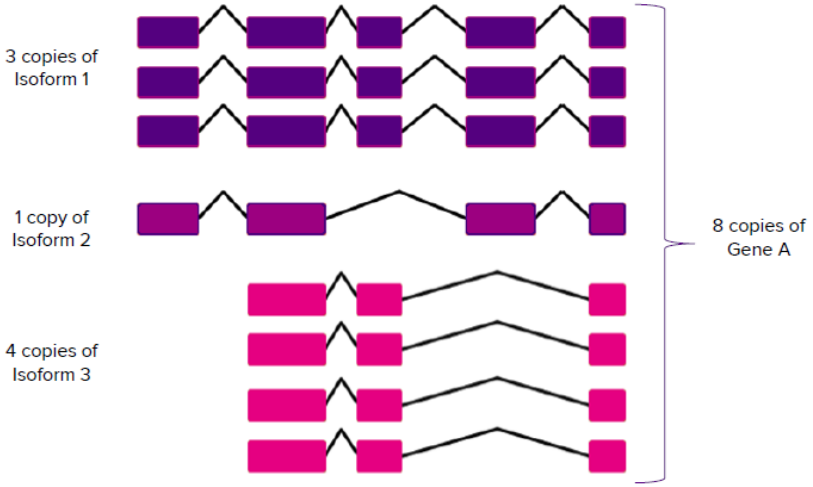
HT IFC Workflow

Sequencing Considerations

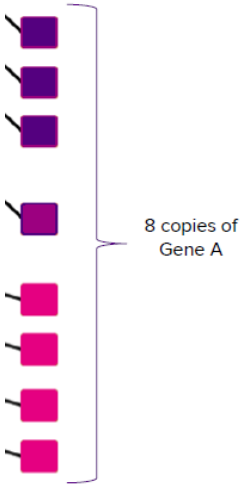
Competitive Update

Full length vs. end counting

96 Cell IFC



HT IFC



Whole transcript is analyzed in sequencing run

- Splice variants
- Allelic expression
- Paired transcripts (i.e. α/β TCR)

Only 5'/3' ends are read/counted

- Relative gene expression only
- Rare cell phenotypes (more cells)

How does the method affect the data?

Full length:

Can be more sensitive (can detect lower starting copy number)

More information (more genes detected)

Wider number of applications (TCR)

3’ End Counting:

Shallower read depth (more cells in one sequencing run)

Sufficient for cell type classification

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Full length chemistry
(96 cell IFC)

SMARTer® template switching

Amplifies full-length cDNA molecules for sequencing – 96 Cell IFC

1) First strand synthesis and tailing by SMARTScribe Reverse Transcriptase

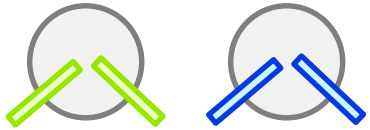
SMARTer IIA Oligonucleotide

2) SMARTer IIA Oligo binds to tail, reverse transcriptase “switches” to primer template and incorporates tag (purple)

3) PCR primers added, amplification of double-stranded cDNA

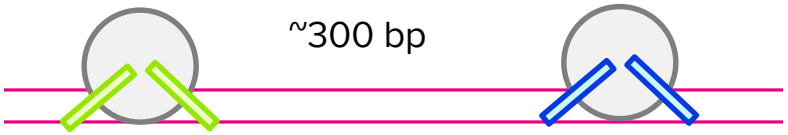
4) Amplify ds cDNA by PCR

Nextera XT Illumina Library Prep



Transposons with specific oligo insert sequences

Amplified cDNA from C1



Transposon cuts and inserts sequences

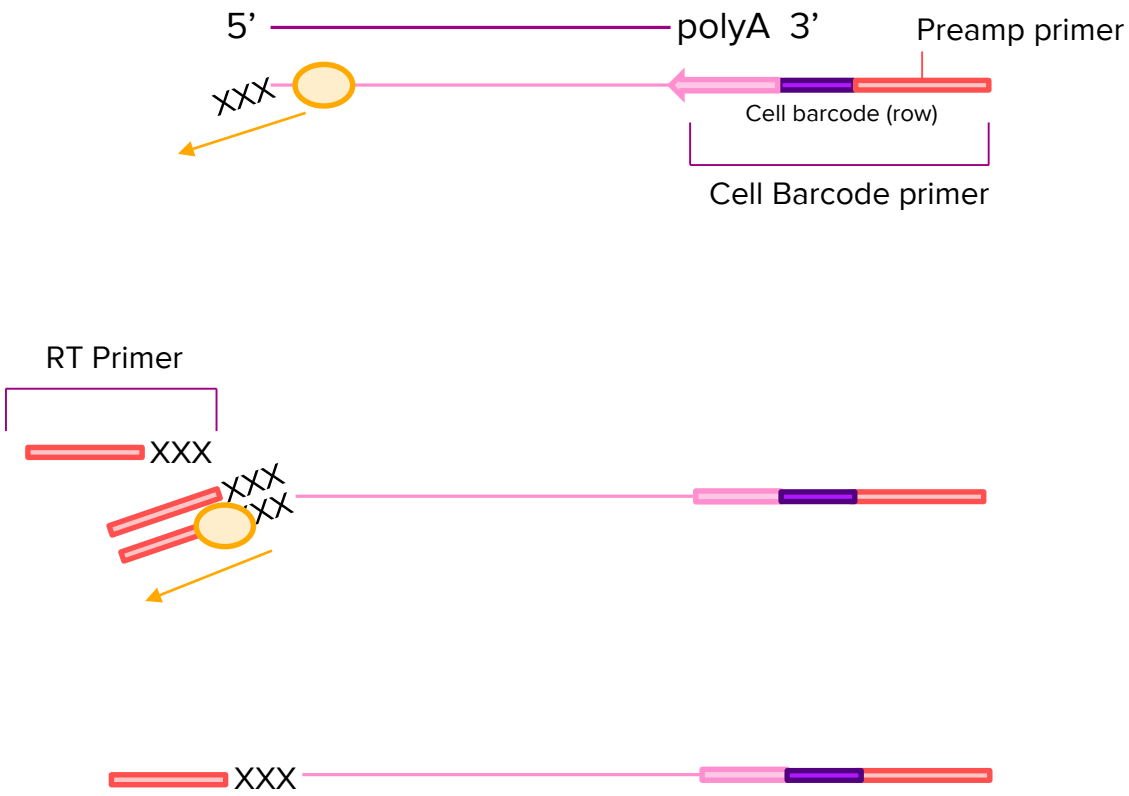
Repair ends

Perform PCR to amplify and to incorporate barcodes and sequencer adaptors

P5 – P5 Illumina Adaptor
BC1 – Index 1
R1 – Read 1 sequencing primer
P7 – P7 Illumina Adaptor
BC2 – Index 2
R2 – Read 2 sequencing primer

3’ End Counting Chemistry
(HT IFC)

Reverse transcription introduces a “row-specific” barcode in the HT IFC



dT primer contains row barcode sequence

Template switch

1st strand cDNA

Preamplification is similar in both methods

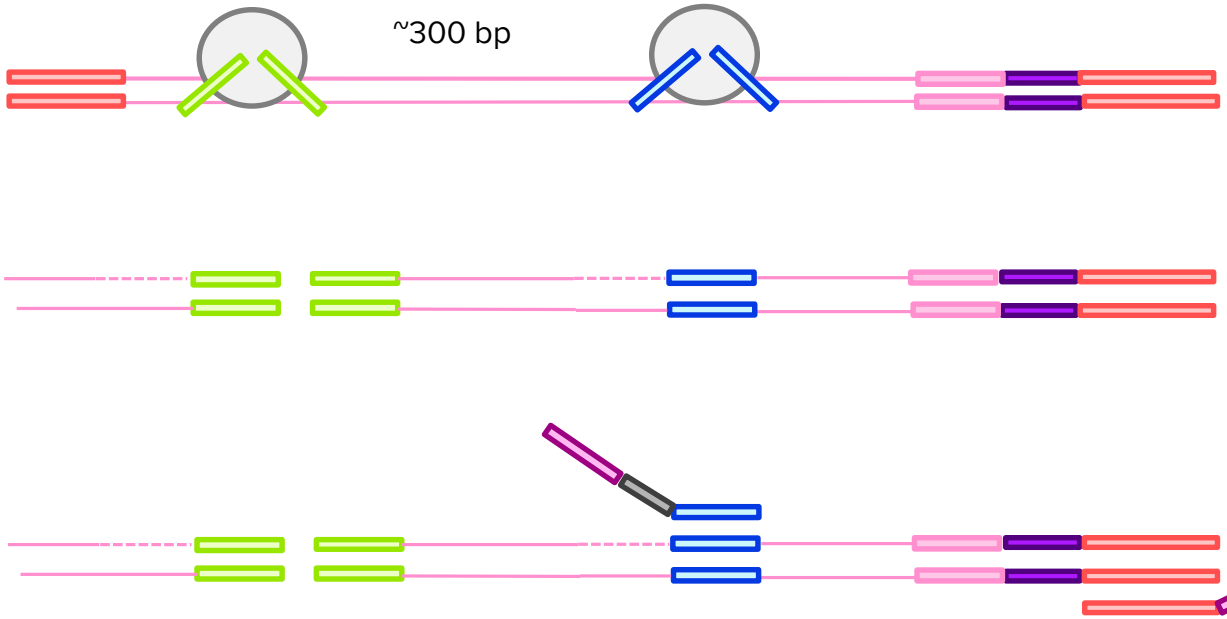
Preamp primer

1st strand cDNA

Preamp primer

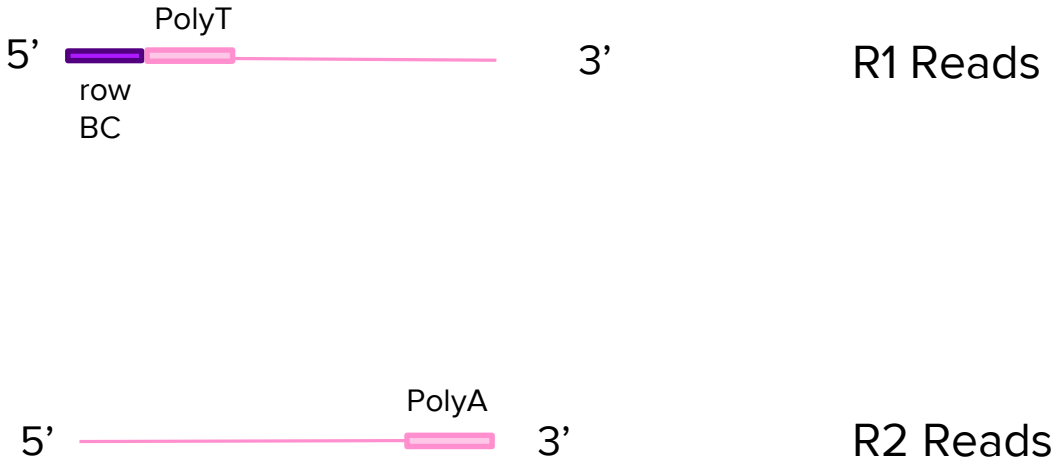
Double-stranded cDNA
(Harvested from C1)

Library preparation uses 3’ end enrichment



P7 – P7 Illumina Adaptor
Nex BC – Nextera i7 column barcode
R2 – Read 2 sequencing primer
Row BC – Row barcode
R1 – Read 1 sequencing primer
P5 – P5 Illumina adaptor

Reads with HT IFC fragments*



*the Column BC (Nextera BC) is read in a separate “Index” read, in between reads 1 and 2

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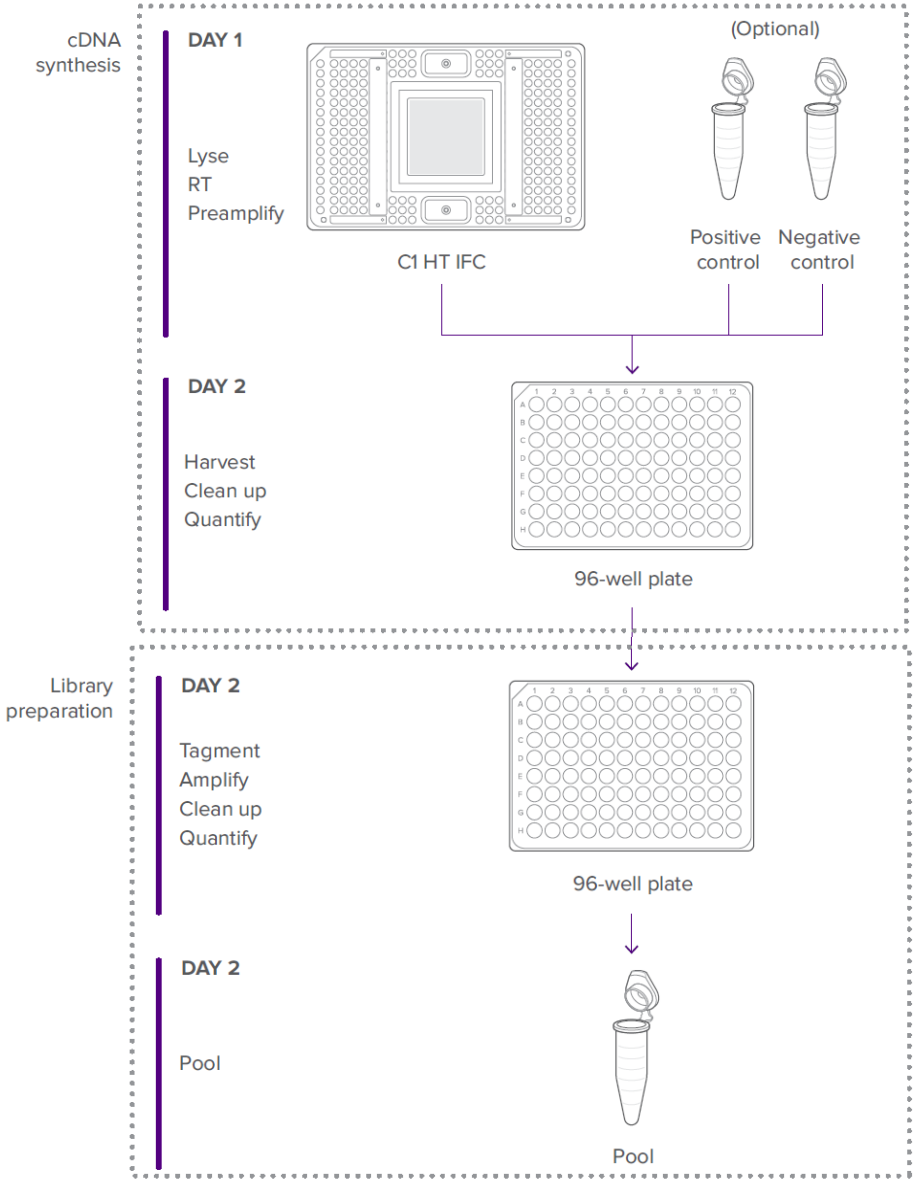
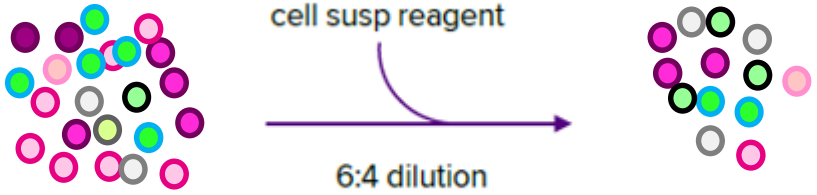
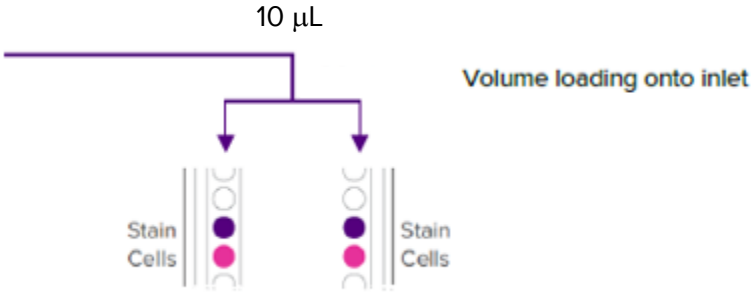
Workflow overview



Cell loading

small cells & medium cells have different recommended starting concentrations





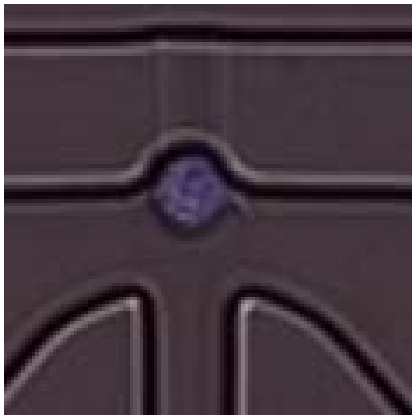
Small cells – 1250 cells/µL
Medium cells – 400 cells/µL

Small cells – 750 cells/µL
Medium cells – 240 cells/µL

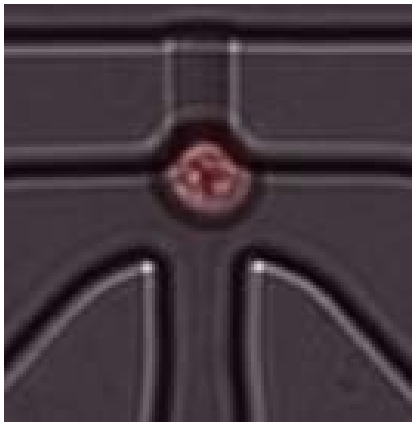
Small cells – 6,750 cells loaded
Medium cells – 2,160 cells loaded

Imaging all 800 capture sites is critical for assessing doublet rate*

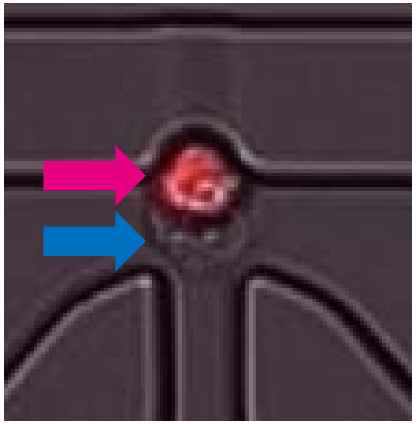
Single blue



Single red



Doublet



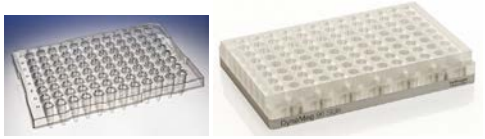
*particularly in stacked configuration

Please resource an automated microscope that can image Hoechst 34580 and Cell Tracker Orange

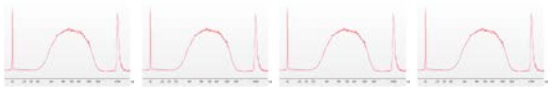
Equimolar library pooling is recommended

Equimolar pooling

Library amplification



Column pool sample cleanup (96-well plate) with magnetic plate



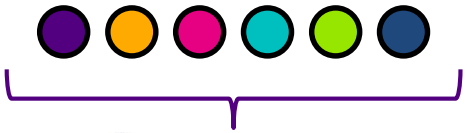
Column pool measurement (Bioanalyzer or Qubit)

Equimolar pooling of column samples (size represents volume)

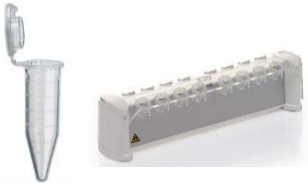
Load sequencer

Equivolume pooling

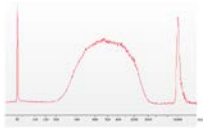
Library amplification



Equivolume pooling of column samples (size represents volume)



Single tube bead cleanup with magnet

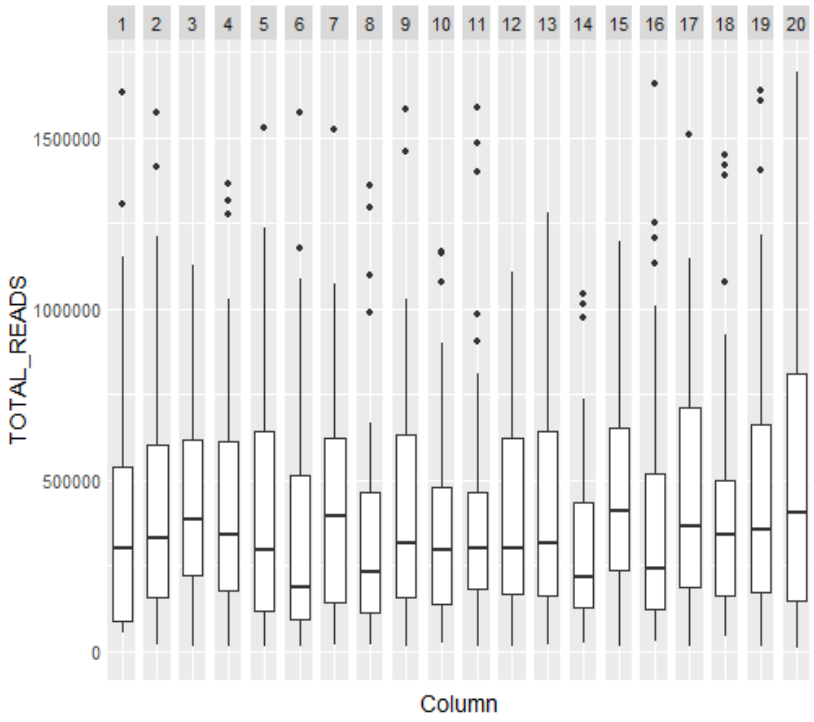


Bioanalyzer

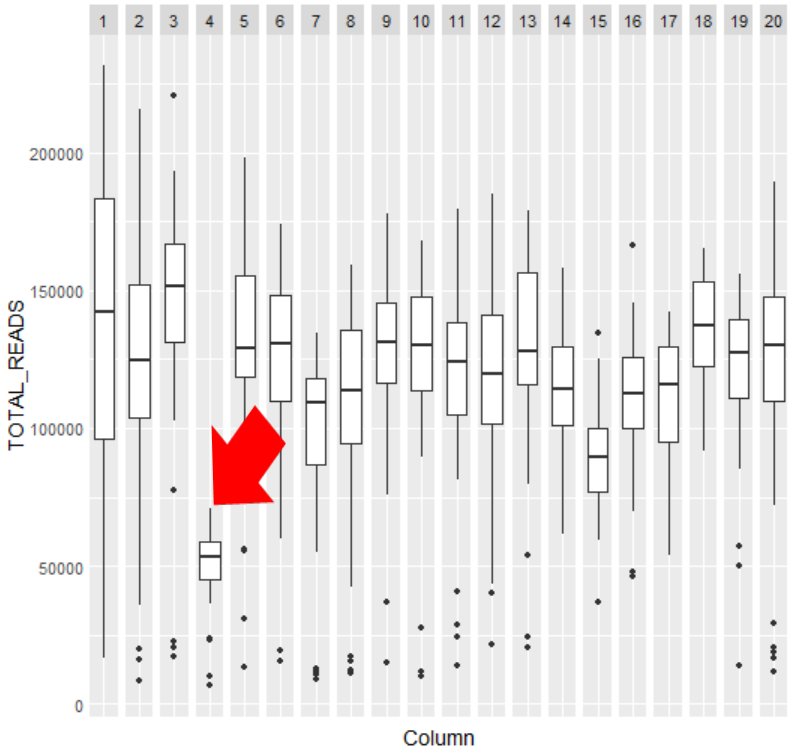
Load sequencer

Equimolar library pooling provides more even read distribution

Equimolar pooling



Equivolume pooling



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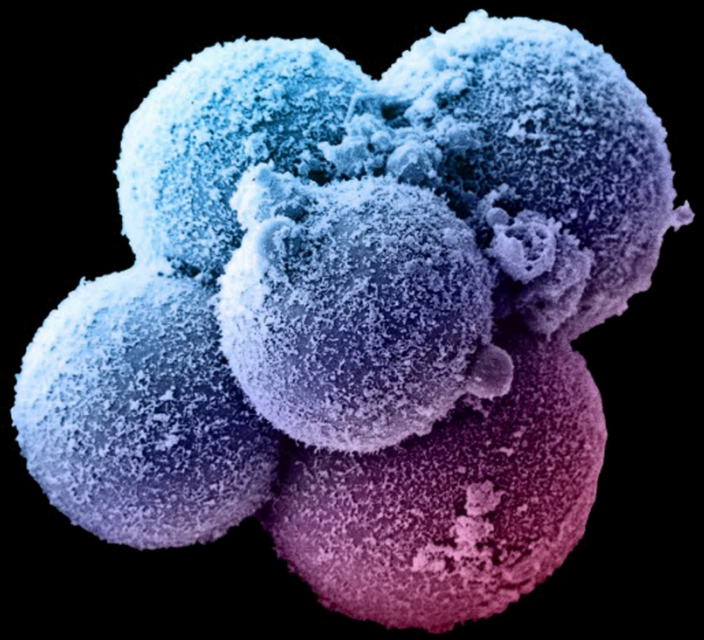
How much read depth do I need?



Comparison of Single-Cell RNA Sequencing methods

‘Power Analysis of Single Cell RNA-Sequencing Experiments’

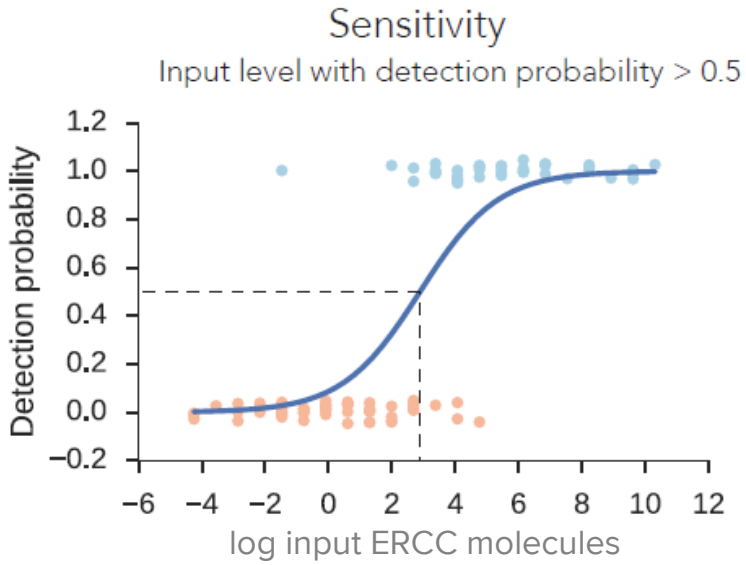
Svensson, V., Natarajan, K.N., Ly, L., Miragaia, R.J., Labalette, C., Macaulay, I.C., Cvejic, A., Teichmann, S.A.

bioRxiv (2016)

Definitions

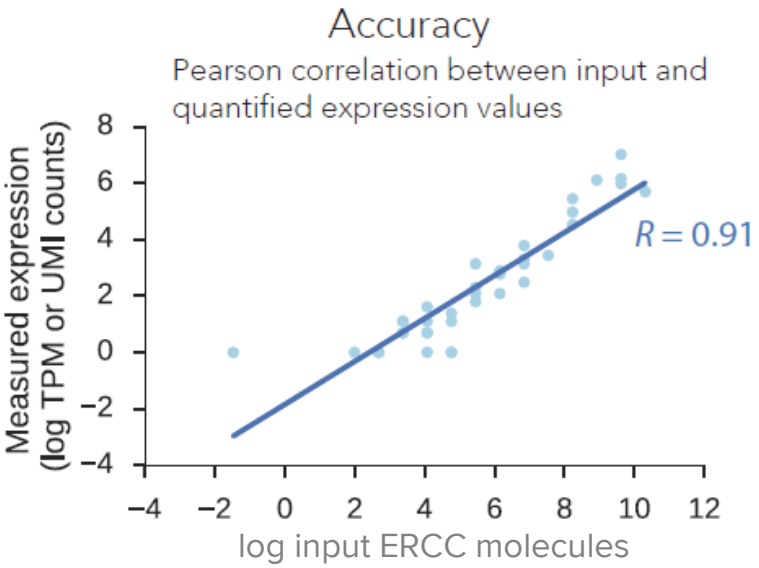
Sensitivity: ability to detect low copy number genes

- Calculated by quantifying the lowest copy number ERCC spike that was detected 50% of the time in each method. Lower = better.

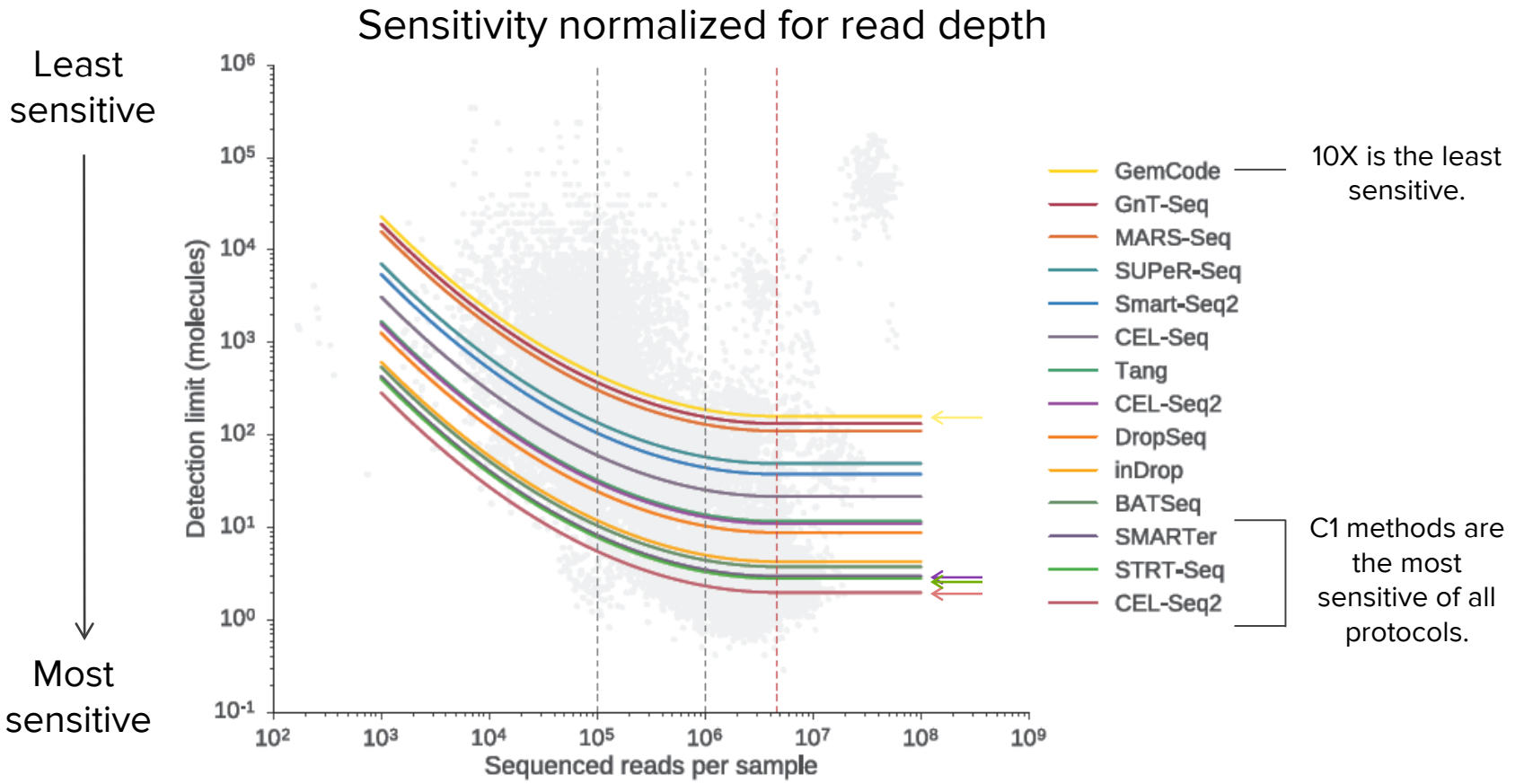


Accuracy: ability to measure changes in gene expression

- Calculated by quantifying known changes in ERCC spike quantities, which are present in 2-fold amounts. Higher = better.
- Could also be interpreted as “linearity”



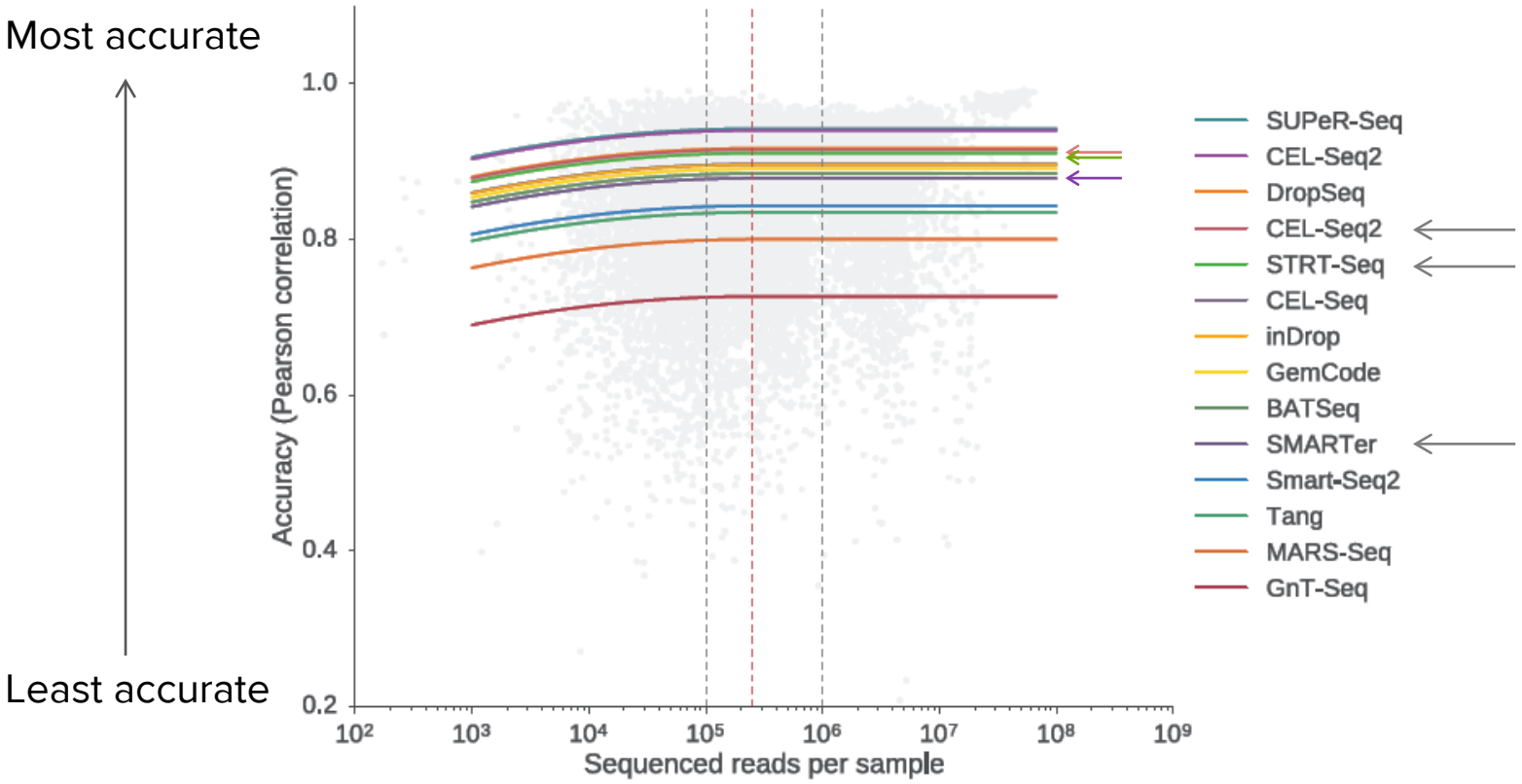
C1 is the most sensitive method



C1 is significantly more sensitive than alternative methods (note log scale).
Optimal read depth is between 100,000 and 1M reads/cell.

- <100,000 reads/cell = far fewer molecules detected
- Few gains beyond 1M reads/cell

Accuracy is good regardless of method or read depth



No major differences in ability to quantify gene expression (note linear scale)

Sequencing depth considerations

250M reads/lane, Hi Seq

Number of HT column pools or 96 cell IFCs	No. of cells	Reads/cell (M)
1	40	6.3
2	80	3.1
1 96 Cell IFC	96	2.6
4	160	1.6
8	320	0.781
4 96 Cell IFC (current max)	384	0.651
10	400	0.625
16	640	0.391
20	800	0.312

Overview

Current C1 96 IFC portfolio

Introducing the HT IFC

Library prep approaches with the Fluidigm C1
3’ end counting vs. Whole transcriptome sequencing

Chemistry
Whole transcriptome – current C1 (96 IFC)
3’ end counting (HT IFC)

HT IFC Workflow

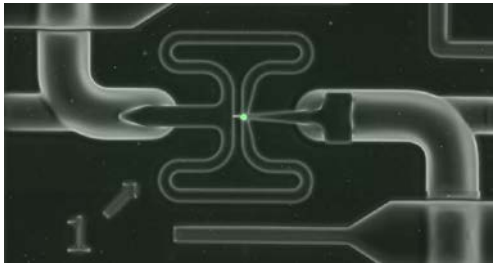
Sequencing Considerations

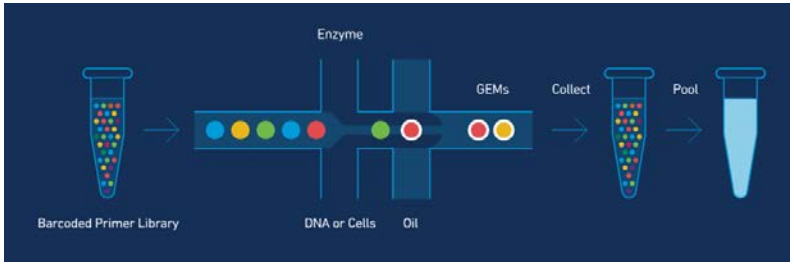
Competitive Update

C1 – major competitors overview

Technology	Method of capture	Cells/Run (low-hi)	Cost/cell range (prep only) (€)	Chemistry
Fluidigm C1 – HT IFC	microfluidics	(400 – 750)	(4.66 – 2.48)	3’ end counting
Drop-Seq (homebrew)	droplets	10,000+	??	3’ end counting
10X Chromium	droplets	(8000-48000)	(1.88 – 0.12)	3’ end counting
Wafergen iCell8	Microwell dispensing	(300-600)	(7.58 – 3.79) USD	3’ end counting
Illumina/Bio-Rad ddSeq	droplets	1000s…	1 USD	3’ end counting
FACS (variable mfg.)	Flow sorting into 384-well	(100 – 1000s)	Variable depending on chemistry	Typically full length, open method

A closer look at Fluidigm vs. 10X

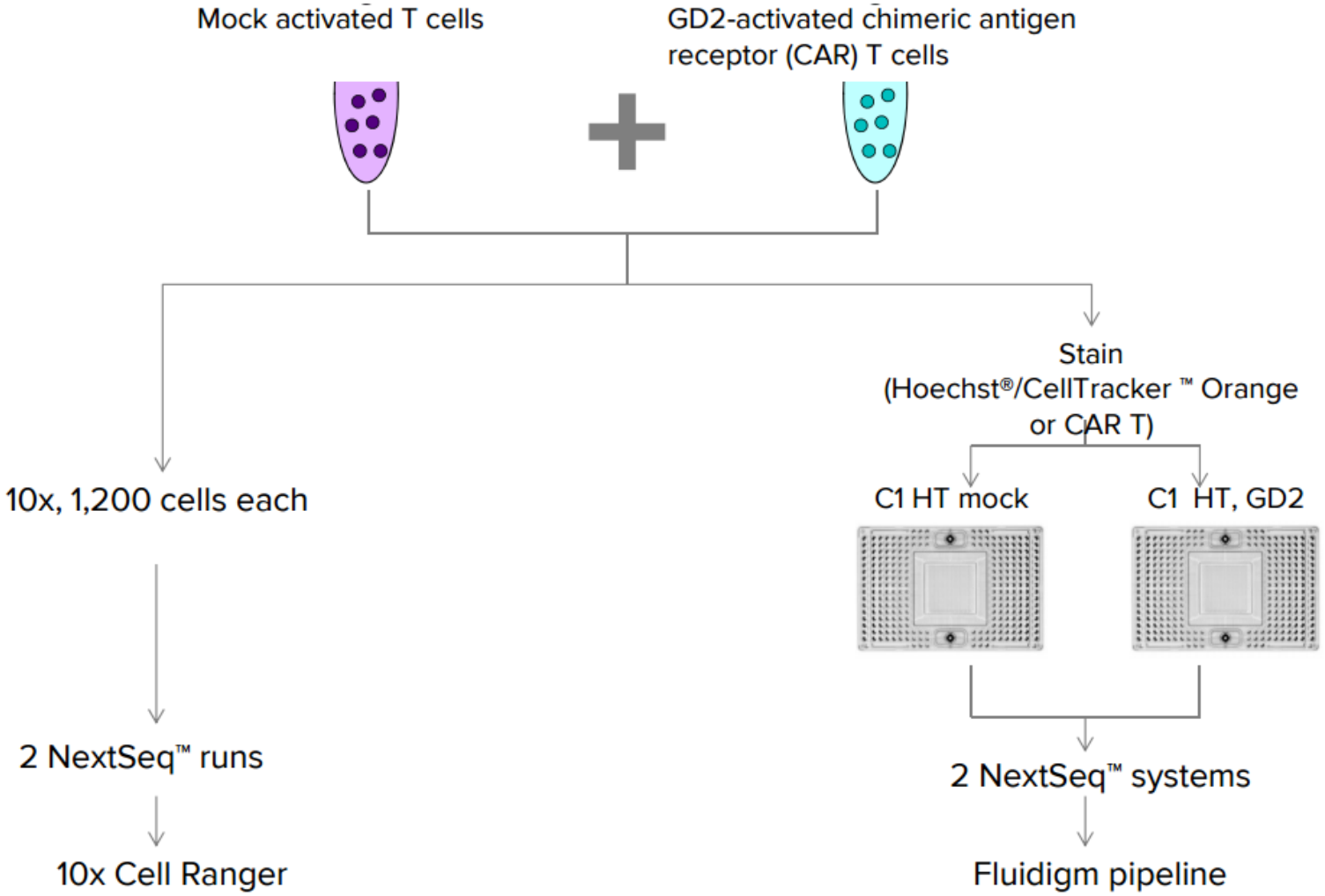




What are you missing?

- More cells is not always more information
- Sensitivity of the chemistry is important to data quality

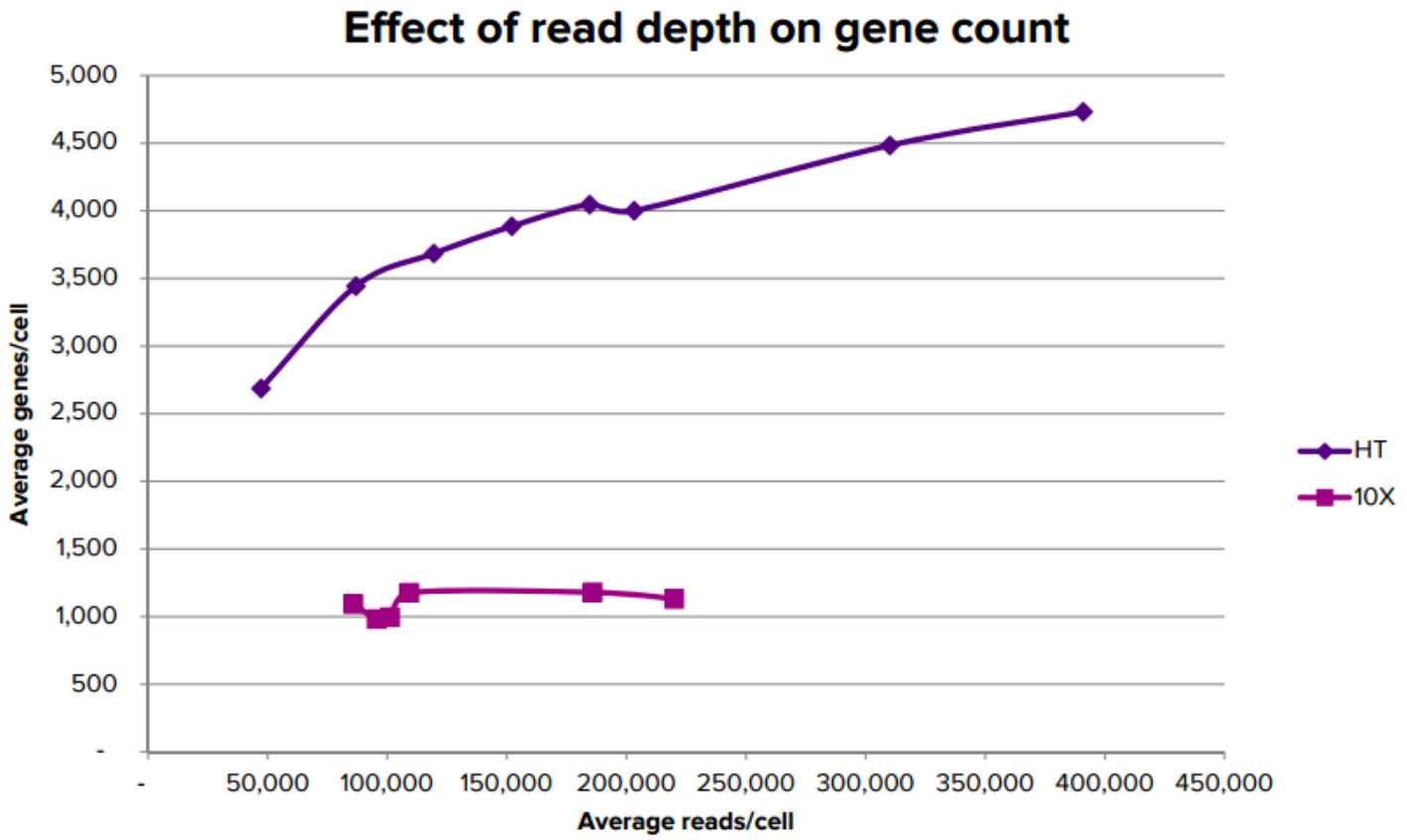
Side-by-side comparison experiment



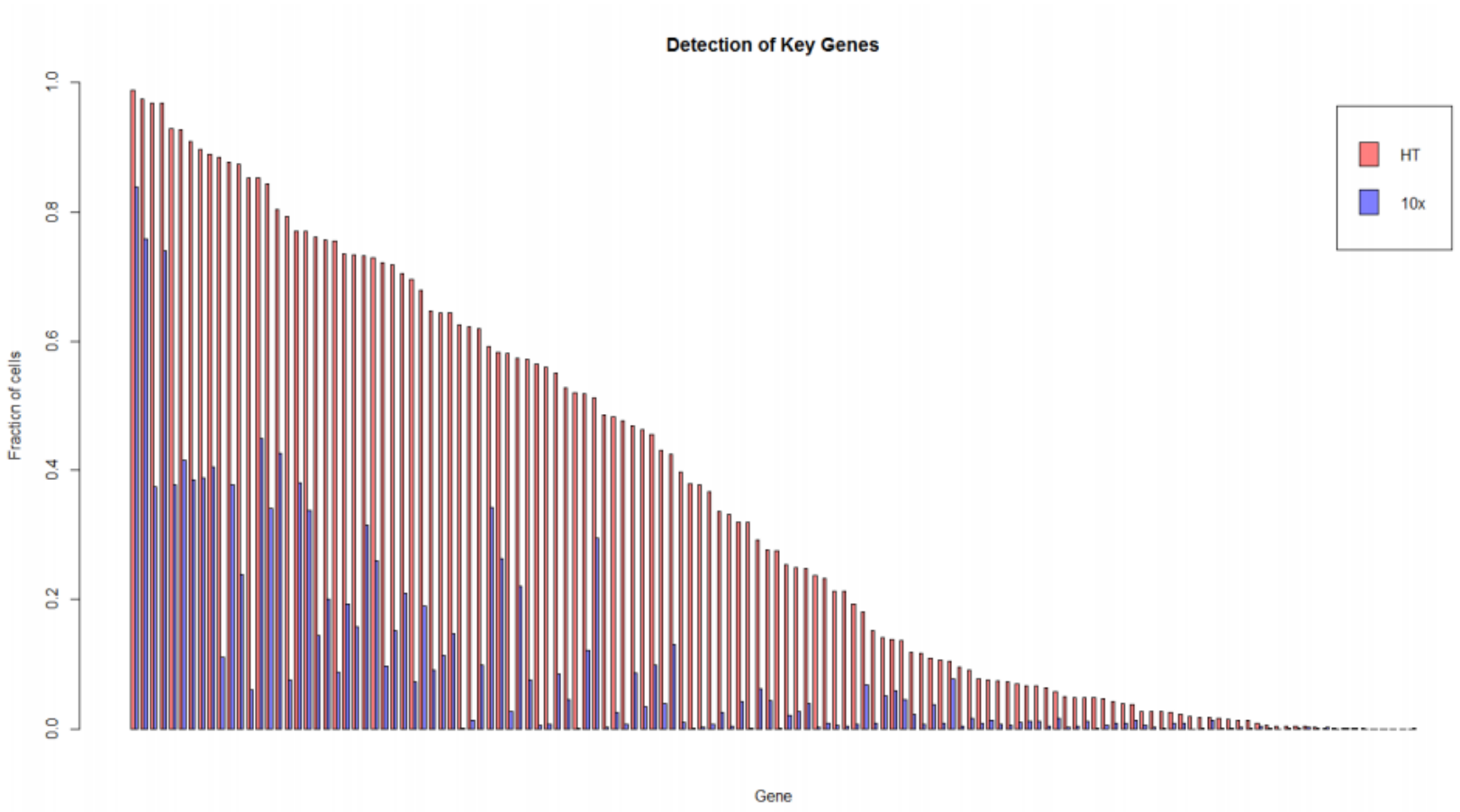
Sequencing metrics

Metric	10x Chromium™	Fluidigm C1 HT	Fluidigm C1 HT (down-sampled)
Number of cells	1,551 CAR T 1,397 mock	529 CAR T 348 mock	529 CAR T 348 mock
Average reads/cell	203,000	499,302	152,000
Total genes detected	15,002/32,732 (46%)	17,797/23,732 (75%)	17,079/23,732 (72%)
Median genes/cell	1,155	4,997	4,029
% reads aligned to transcriptome	65%	53%	53%
% intronic	16%	9%	9%
% exonic	65%	74%	74%
% intergenic	3%	17%	17%

C1 HT produces many more genes than 10x Chromium



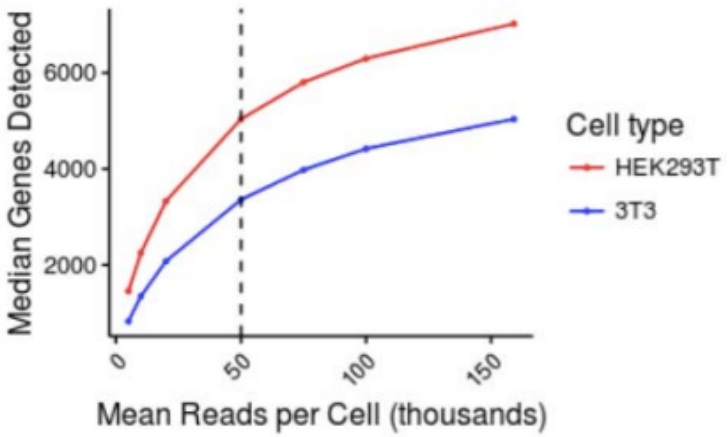
C1 HT detected T cell-specific genes in a larger fraction of cells

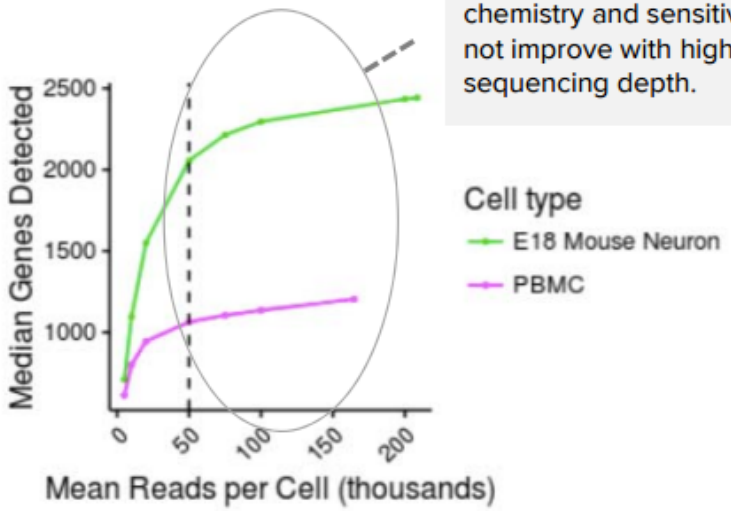


New announcement ASHG 2016

Claim	Specs
New Instrument	• Same technology • $50k per unit (anecdotal information about promo vs. list price)
Expanded scale and throughput	• Refined microfluidics • Delivers 500-10,000 cells/channel (@ 8 channels = 4,000 – 80,000 cells per run) • Same doublet rates (0.9 % per 1000 cells to ~5.9% for 5000 cells or higher)
New reagent kit	• Detects 6k-7k genes/cell at 100k reads (lower for T cells) • Shipping in H1 FY17
New applications (in development)	• SC Targeted TCR seq • Full length V(D)J with both a/b chains • No launch date available,

New v2.0 chemistry does not yield major gains for all sample types





Performance is similar to v1.0 chemistry and sensitivity does not improve with high sequencing depth.

Why chose the C1?

C1 RNA Seq HT offers several performance advantages over 10x Genomics:

- Higher sensitivity to detect more genes, including low-to-mid-abundant transcripts

- More robust assay performance to detect biologically relevant genes in a greater number of cells

Summary

Medium HT IFCs are available

Small HT IFCs are still in development

C1 offers application and throughput flexibility

C1 has superior data quality when compared with competitors

Thank you.