C1 mRNA Sequencing

Rapidly characterize heterogeneity, identify critical cell populations.

Individual cells are unique—they differ by size, protein levels, and expressed mRNA transcripts. Discovering the cell populations that drive cancer development, activate differentiation, and modulate biological mechanisms requires single-cell resolution and comprehensive techniques to discover and characterize cell types and estimate their population frequency. With the C1™ single-cell mRNA sequencing (C1 mRNA Seq) workflow, you can accomplish all of this while streamlining your path to new discovery.

Now, you can rapidly and reliably isolate, process, and profile individual cells at any scale. With over 40 studies published since its launch in 2012, C1 is the only vetted technology available for single-cell analysis. With one system, you can expansively explore the transcriptome at any scale—from small pilot studies to discover novel isoforms or gene signatures, then scale up to large survey studies to quantitate gene expression and determine cell population frequencies.

Features

- Rapidly process up to 1,600 cells per day
- Full transcript or 3′ end-counting chemistry allows you to maximize your sequencing capacity
- Easy, integrated workflow

	C1 mRNA Seq	C1 mRNA Seq HT
Throughput	96 cells	800 cells
Methodology	Full transcript or 3′ end-counting	3′ end-counting
Chemistry	SMARTer® V1	SMARTer V3
Run time	10 hours	6.5 hours
Hands-on library preparation time	1 hour	20 minutes
On-IFC barcoding and multiplexing	No	Yes
Cell Imaging	Stain and visualize	Stain and visualize
Cell load structure (port x cells captured)	1 x 96	2 x 400

Workflow

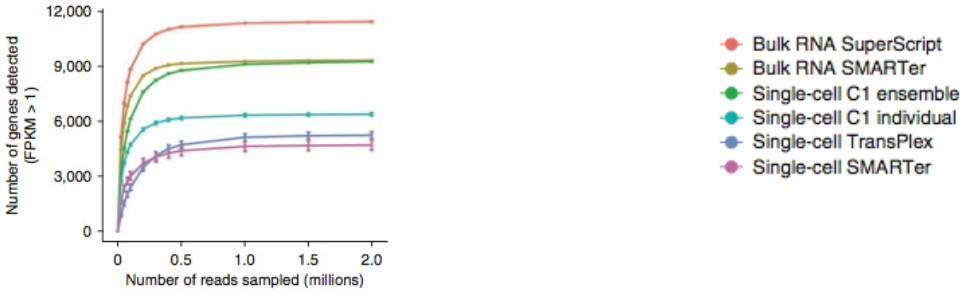
Perform pilot studies or large survey studies with one easy, load-and-go workflow.

Isolate	Stain & Visualize	Reverse Transcribe	Harvest	Prepare Libraries	Data Analysis
Individualize 96 or 800 cells	Verify cell state	Generate cDNA and amplify template	Collect single-cell cDNA template	Prepare for sequencing off-IFC	Visualize your cell populations with Singular™

C1 mRNA Seq—the gold standard

In their 2013 *Nature Methods* publication, "Quantitative assessment of single-cell RNA-sequencing methods," Wu et al. were the first to qualify the robust performance of C1 mRNA Seq against alternate gene expression methods.

a



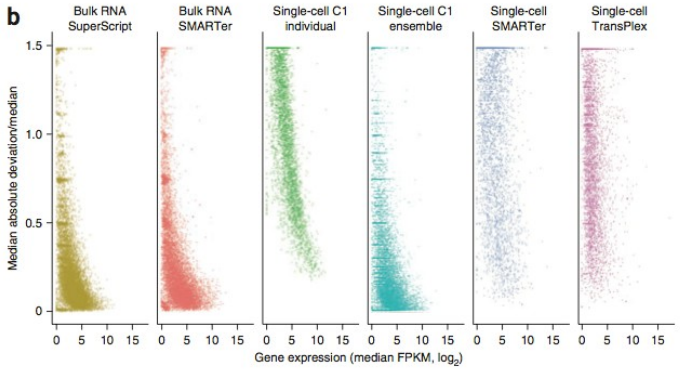


Figure 1. a) Gene expression saturation curves for various sample preparation methods demonstrate the C1 system's ability to detect the maximum number of genes within single cells in this study. When genes detected in individual cells are informatically ensembled and averaged, the genes detected are comparable to those in bulk, tube based methods. b) Variation in gene expression across sample replicates for each preparation method illustrates the heterogeneity present within single cells. Again, the C1 system's single-cell ensemble is representative of bulk results.

Get a global view of cellular heterogeneity

Single-cell mRNA sequencing provides you with a complete picture of cellular heterogeneity and the signatures defining specific cell populations. Now, you can prepare hundreds to thousands of cells with C1 and rapidly analyze your sequencing data with Singular to discern novel subpopulations. In their 2014 *Nature Biotechnology* publication, “Low-coverage single-cell mRNA sequencing reveals cellular heterogeneity and activated signaling pathways in developing cerebral cortex.”, Pollen et al. used this approach and discovered new neural markers for specific cell types that were not discernable in bulk tissue.

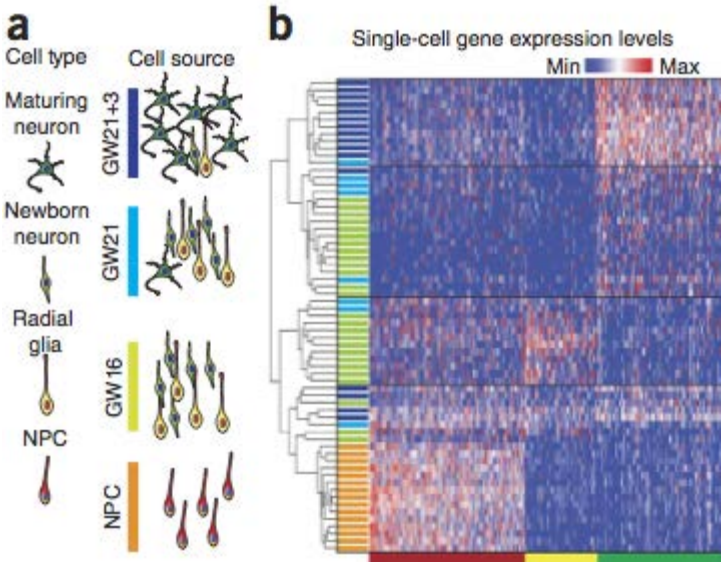


Figure 2. Hierarchical clustering of single cells selected from different stages of neuronal differentiation reveal four major groups of cells and candidate markers PDZRN3, NMT, CAMKV, ADRA2A, CKS2, and HMGB2

Maximize your discovery with C1 mRNA Seq HT

As we discover more novel, less abundant cell populations, there is growing demand to expand single-cell studies to the 10,000s and 100,000s of cells. By increasing the magnitude of our experimental models, we can discern tissue structure, uncover new cell types, and understand population frequency.
C1 mRNA Seq HT lets every C1 user scale their studies to the 10,000s of cells and maximize each sequencing run without investing in new equipment. This simple workflow can perform two IFC runs per day enabling you to process up to 1,600 cells in 24 hours. Additionally, this workflow streamlines library preparation with on-IFC multiplexing— reducing library preparation costs by 40x and increasing capture efficiency by 2x, while providing you with the most efficient workflow to date.

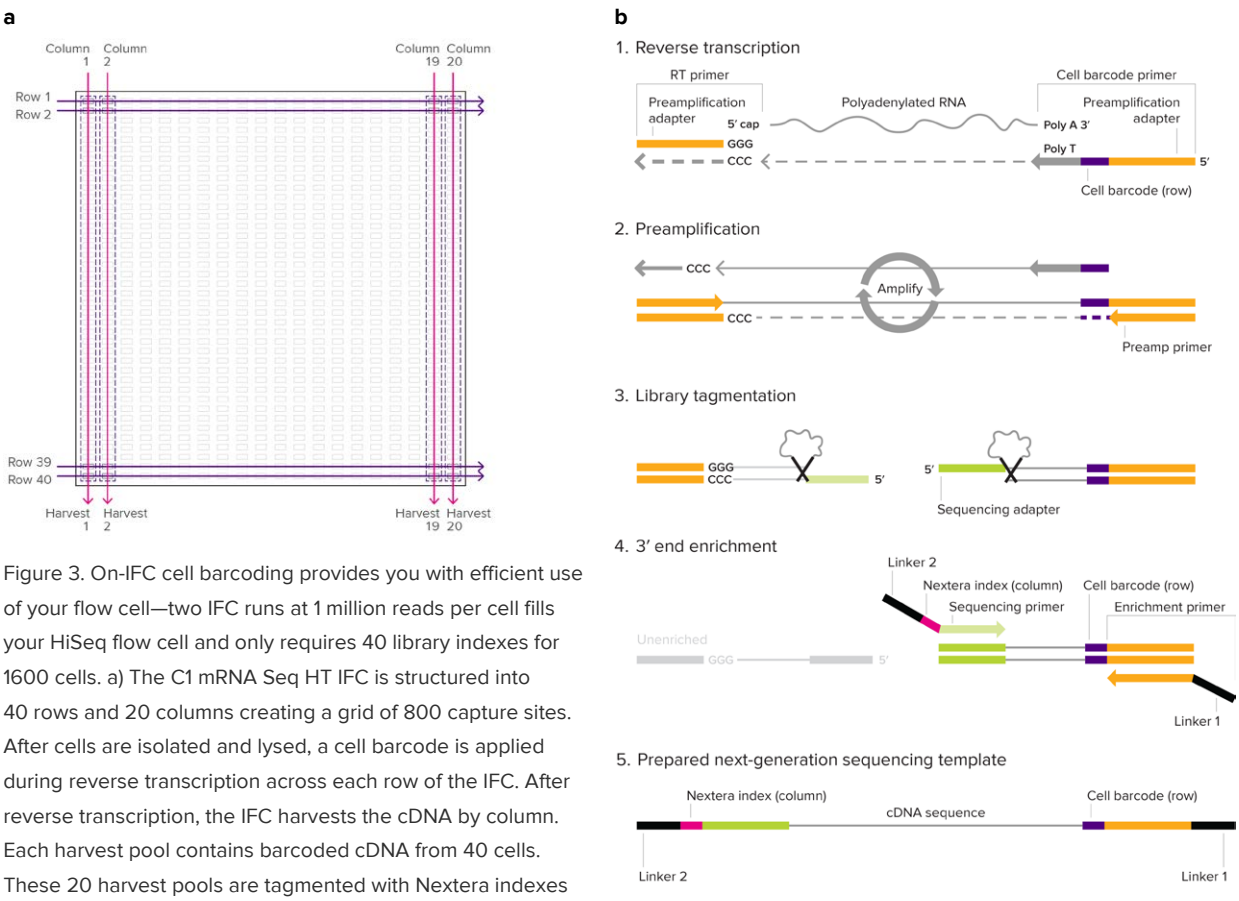


Figure 3. On-IFC cell barcoding provides you with efficient use of your flow cell—two IFC runs at 1 million reads per cell fills your HiSeq flow cell and only requires 40 library indexes for 1600 cells. a) The C1 mRNA Seq HT IFC is structured into 40 rows and 20 columns creating a grid of 800 capture sites. After cells are isolated and lysed, a cell barcode is applied during reverse transcription across each row of the IFC. After reverse transcription, the IFC harvests the cDNA by column. Each harvest pool contains barcoded cDNA from 40 cells. These 20 harvest pools are tagmented with Nextera indexes for sequencing. b) This detailed diagram illustrates how cell barcodes are integrated and Nextera indexes are applied. The finished product contains a Nextera index and a cell barcode, uniquely identifying every cell.

Discovery to validation with C1 and Biomark HD

With C1 and Biomark™ HD you have a complete workflow to discover novel isoforms and determine cell population signatures and then target specific genes with Biomark to verify the biological pathway and gene function.

Discover	Survey	Validate	Analyze
Identify novel transcripts with C1 mRNA Seq	Understand prevalence with C1 mRNA Seq HT	Use known signatures to verify results with C1 and Biomark	Aggregate and visualize your data with Singular 3.0

Specifications

	C1 mRNA Seq	C1 mRNA Seq HT
Sample sources	Primary and cultured cells	
Cost per cell	$$	$
IFC availability	C1 mRNA Seq IFC (5–10 µm) C1 mRNA Seq IFC (10–17 µm) C1 mRNA Seq IFC (17–25 µm)	C1 mRNA Seq HT IFC (10–17 µm) Coming soon: 5–10 µm IFCs
Sample Input	200–1,000 cells	2,000–5,000 cells
Small-cell (5–10 µm) IFC cell capture efficiency	≥81% of capture sites will contain cells with 1,000 HL60 cells input[1]	
Medium-cell (10–17 µm) IFC cell capture efficiency	≥90% of capture sites will contain cells with 1,000 K562 cells input[1]	≥90% of capture sites will contain cells with 5,000 K562 cells input
Large-cell (17–25 µm) IFC cell capture efficiency	≥90% of capture sites will contain cells with 1,000 BJ Fibroblast cells input[1]	
Supported platforms	Illumina® HiSeq® and MiSeq® platforms	
Time to result	Cells to sequence-ready library: 13 hours	
Hands-on time	<3 hours	
Typical sequencing performance[2]		
Number of detected RefSeq genes with TPM >1[3]	≥4,000	≥4,000
Reads mapped to rRNA/total reads	≤1% rRNA/total reads	≤3% rRNA/total reads

1 Across a variety cell types between 5 and 25 µm, with cells run in the correct size IFC, we observe that on average >90% of occupied capture sites contain single cells.

2 Sequencing performance for K562 cell line was evaluated with C1 mRNA Seq IFC (10–17 µm), C1 mRNA Seq HT IFC (10–17 µm), and C1 mRNA Seq IFC (17–25 µm). Performance for HL60 cell line was evaluated with the C1 mRNA Seq IFC (5–10 µm). Performance of diverse cell types may vary depending upon the quantity of available RNA template, variability in expression levels, and cell preparation conditions.

3 Observed with ≥200,000 total single-end reads or ≥400,000 total paired-end reads.

Learn more

For more information about Fluidigm applications for single-cell genomics and C1, visit fluidigm.com.

CORPORATE HEADQUARTERS

7000 Shoreline Court, Suite 100
South San Francisco, CA 94080 USA
Toll-free: 866 359 4354 in the US
Fax: 650 871 7152
fluidigm.com

SALES

North America | +1 650 266 6170 | info-us@fluidigm.com
Europe/EMEA | +33 1 60 92 42 40 | info-europe@fluidigm.com
China (excluding Hong Kong) | +86 21 3255 8368 | info-china@fluidigm.com
Japan | +81 3 3662 2150 | info-japan@fluidigm.com
All other Asian countries | +1 650 266 6000 | info-asia@fluidigm.com
Latin America | +1 650 266 6000 | info-latinamerica@fluidigm.com

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