Chromium™ Single Cell 3’ Solution v2

A New Standard for Single Cell RNA-Seq

Revision A, November 2016
## The Chromium™ Single Cell 3’ Solution

<table>
<thead>
<tr>
<th>Chromium™ Controller</th>
<th>Chromium Single Cell 3’ v2 Consumables</th>
<th>Cell Ranger™ Pipelines</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Chromium™ Controller" /></td>
<td><img src="image" alt="Chromium Single Cell 3’ v2 Consumables" /></td>
<td><img src="image" alt="Cell Ranger™ Pipelines" /></td>
</tr>
</tbody>
</table>
| - GemCode™ Technology  
- Automated  
- Flexible throughput: from a hundred to a million cells | - Chip for single cell partitioning in GEMs  
- Reagents for RT, amplification and library construction | - Informatics solution for single cell expression profiling  
- Pre-processing, QC and analytics |
Single Cell Analysis: Biology in HD

Bulk

“Tumor”

“Blood”

Single Cell Resolution

- Cancer stem cells
- Stromal cells
- Tumor-infiltrating lymphocytes

- Platelets and red blood cells
- Immune cells
- Circulating tumor cells
Single Cell Partitioning in GEMs

- Cell
- Gel Bead with Barcoded RT Primers
- RT Reagents in Solution
- Partitioning Oil
High Diversity Barcode Library

- ~750,000 Discrete Reagents in One Tube
- Defined barcode sequences
- Highly uniform size and barcode representation
- Built-in sequencing adapter, barcode and primer
Single Cell Partitioning, Lysis and Barcoding
Single Cell 3’ Digital Gene Expression

- Rapid partitioning and lysis of cells in < 7 minutes
- Low cell loss
- No lower limit on cell size

Output: Digital gene expression profiles from every partitioned cell
Rapid and Efficient Microfluidics

- Partitions 100 - 10,000+ cells per channel in < 7 minutes
- Recovers ~65% of all loaded cells
- Low doublet rate: 0.9% per 1,000 cells
Human-Mouse Mixtures Confirm Predicted Doublet Rates

1:1 Mixture of Human (293T) and Mouse (NIH/3T3) Cells, sequenced to ~30-60K reads/cell

- ~150 loaded cells
- 100 recovered cells
- 0 doublets* (0.0%)

- ~1,530 loaded cells
- 1,015 recovered cells
- 6 doublets* (0.6%)

- ~10,000 loaded cells
- 6,806 recovered cells
- 345 doublets* (5.1%)

- ~19,000 loaded cells
- 13,096 recovered cells
- 1,370 doublets* (10.5%)

* Includes observed (human-mouse) and inferred (human-human, mouse-mouse) doublets
- Standard sequencing configurations
- Easier to multiplex with non-SC libraries
- High quality UMI and Cell Barcode reads
- High performance on patterned flowcells
One Day Workflow

- Optimized reverse transcription and cDNA clean-up
- Enzymatic fragmentation
- Cell suspension to library in 1 day
v2 Reagents and Workflow Boosts Sensitivity

293T Human Embryonic Kidney Cells

NIH/3T3 Mouse Fibroblasts
Choose the optimal sequencer for the scale of your experiments

HiSeq 4000 enables sequencing of 10,000s-100,000+ cells per flow cell
Cell Ranger™ – Informatics Workflow

- Complete Linux-based software package for single cell analysis
- Bundled with STAR for efficient transcriptome alignment
- Outputs are standard formats plus Loupe visualization
- Runs anywhere: Local Mode and Cluster Mode
Cell Ranger™ 1.2 Updates

- Flexible demultiplexing features
- Built-in differential gene expression, PCA, t-SNE and clustering analysis
- New functionality for aggregating data from multiple sequencing runs, libraries and/or samples
- High performance and scalability: Enables analysis of 1,000,000+ cell datasets
Million-scale Single Cell Analysis

- E18 Mouse Cortex, Hippocampus And Ventricular Zone
- 17 Chromium SC v2 chips
- 136 ~10,000 cell libraries
- 4 HiSeq 4000 flow cells
- Cell Ranger 1.2

1,308,421 Single Cell Expression Profiles
3D Representation of 1,308,421 Single Cells from Mouse Brain
### Chromium Single Cell 3’ Solution Pricing

<table>
<thead>
<tr>
<th>Product</th>
<th>Units</th>
<th>List Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromium™ Controller</td>
<td>1 Instrument</td>
<td>$125,000</td>
</tr>
<tr>
<td>Chromium Single Cell Controller</td>
<td>1 Instrument</td>
<td>$75,000+</td>
</tr>
<tr>
<td>Chromium Single Cell 3’ Reagent Kit v2</td>
<td>16 reactions</td>
<td>$20,000*</td>
</tr>
<tr>
<td>Chromium™ i7 Multiplex Kit</td>
<td>96 reactions</td>
<td>$768</td>
</tr>
<tr>
<td>Chromium Single Cell 3’ Chip Kit v2</td>
<td>48 samples</td>
<td>$1,440</td>
</tr>
<tr>
<td>Platform Assurance Plan</td>
<td>12 months</td>
<td>$12,500</td>
</tr>
</tbody>
</table>

*Price per cell $0.13 to $1.20
Chromium™ Single Cell Controller

- A Single Cell Solution for every lab
- Supports Chromium Single Cell 3’ v1 and v2
- Same throughput and dynamic range as the general Chromium Controller
- $75,000
Appendix

GemCode (v1) and Chromium (v1.1) Single Cell 3’ Solution data
Single Cell 3’ Application Highlights

Dynamics of lymphocyte activation in graft-versus-host-disease
Cole Trapnell, Scott Furlan – U. of Washington

Unsupervised identification of immune cell types from 33,000 PBMCs
Rahul Satija – New York Genome Center

Monocle 2: Single cell trajectory analysis
http://cole-trapnell-lab.github.io(monocle-release)/

Seurat: R Toolkit for Single Cell Genomics
http://satijalab.org/seurat/

Nature Webcast:
www.10xgenomics.com/event/single-cell-webinar-cole-trapnell/

Nature Webcast:
www.10xgenomics.com/event/single-cell-webinar-dr-rahul-satija/
Single cell analysis of leukemia before and after bone marrow transplants
With Jason Bielas – Fred Hutch

AML027
(post-transplant)

14% donor

86% host

Grace X.Y. Zheng: Massively parallel digital transcriptional profiling of single cells
ASHG 2016 Podium Presentation
Example 1: Validation of Single Cell Behavior

- 1:1 mixture of ~1,400 human (HEK293T) and mouse (NIH3T3) cells
- 99.4% of cell-occupied GEMs yielded reads mapping to only one species
- 1% inferred doublet rate*

*includes unobserved human:human and mouse:mouse doublets
Number of cells detected: ~1400 cells, Number of raw reads per cell: ~130k
Example 2: Cell Cycle Phases

- Proliferating HEK293T cells were profiled and scored for expression of markers associated with each major cell cycle phase
- Cells from all phases were identified

Combined Expression of Known Phase Markers

HEK293T Cells Ordered by Inferred Cell Cycle Phase

Phase-specific genes derived from Whitfield et al., 2002
Number of cells detected: ~400 cells, Number of raw reads per cell: ~40k
Example 3: Breast Cancer Heterogeneity

Unbiased Automatic Clustering of Three Breast Cell Lines

HER2 Expression Matches Expected Cell Line Status

Number of cells detected: ~1000 cells, Number of raw reads per cell: ~40k
Example 4: Identifying Rare Cell Types

- Jurkat and Raji cells were combined at 9:1, 99:1 and 199:1 ratios and then profiled
- The minority Raji populations were identified in all three mixtures

Number of cells detected: ~1000 cells, Number of raw reads per cell: ~60k
Example 5: Primary Cell Populations

Peripheral Blood Mononuclear Cells (PBMC)

- A complex mixture of different cell types
- Well-studied and readily available primary cells
More structures with increasing # of PBMCs

Bulk RNA-Seq

TSNE1

TSNE2

4,500 PBMCs

16,000 PBMCs

68,000 PBMCs
High reproducibility between channels

- Libraries from 8 channels with a target cell load of ~8000 cells each were combined into one meta sample
- High reproducibility between channels

68,000 PBMCs
Markers highlight distinct sub-populations

- **CD3D, T Cells**
- **FTL, Monocytes**
- **GNLY, NK Cells and T Cell subset**
- **CD79A, B Cells**
Reference populations classify cell types

PBMCS → bead purified → Purified cells → Single cell RNA-Seq

- CD14+ Monocytes
- Dendritic Cells
- CD56+ NK
- CD4+ T Helper 2
- CD4+/CD25+ T Reg
- CD4+/CD45RO+ T Memory
- CD4+/CD45 RA+/CD25- Naive T
- CD8+/CD45RA+ Naive Cytotoxic T
- CD8+ Cytotoxic T
- CD34+
- CD19+ B Cells
Reference populations classify cell types

PBMCS → bead purified → Purified cells → Single cell RNA-Seq

Can select cells from an impure population

Pure CD19+/IgD+ B Cells

80% Pure CD19+/CD27+ B Cells

40% Pure CD34+ Cells

TSNE1
TSNE2

TSNE1
TSNE2

TSNE1
TSNE2

TSNE1
TSNE2

T cells

cd74
0
1
-1
-2

cd3d
0
1
2
3
4
5
Major populations of PBMCs are detected
Major populations of PBMCs are detected

<table>
<thead>
<tr>
<th>Population</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ T</td>
<td>28.4%</td>
</tr>
<tr>
<td>CD14+ Monocytes</td>
<td>5.3%</td>
</tr>
<tr>
<td>CD56+ NK</td>
<td>13.5%</td>
</tr>
<tr>
<td>CD45 RA+ Naïve T</td>
<td>26.4%</td>
</tr>
<tr>
<td>CD19+ B</td>
<td>5.5%</td>
</tr>
<tr>
<td>CD8+ T</td>
<td>18.7%</td>
</tr>
<tr>
<td>Dendritic</td>
<td>1.9%</td>
</tr>
<tr>
<td>CD34+ Progenitors</td>
<td>0.3%</td>
</tr>
<tr>
<td>CD14+ Progenitors</td>
<td>5.3%</td>
</tr>
</tbody>
</table>
Unbiased profiling of frozen PBMCs

Frozen PBMCs, immediately thawed, then performed single cell RNA-Seq

Healthy PBMCs, cryopreserved in 2014

# of cells: 6k, # of raw reads/cell: 36k
Myeloid expansion in AML and CML

AML PBMCs, cryopreserved in 2015

Frozen PBMCs were immediately thawed and profiled
Expansion of myeloid progenitors (CD34+) (44%)

# of cells: 6500, # of raw reads/cell: 38k

CD34+ Myeloid Progenitors

CD14+ Monocytes

CML PBMCs, cryopreserved in 2005 (10 years old)

Frozen PBMCs were sorted and viable cells were profiled
Expansion of monocytes (45%)

# of cells: 900, # of raw reads/cell: 90k

CD34+ Myeloid Progenitors

CD14+ Monocytes
Single cell profiling enables more sensitive comparative analysis

Normal PBMCs vs. AML PBMCs

Significant gene sets

Upregulation of *HPGDS, CD34, KIT*

Upregulation of *SOCB2 & LAPTM4B*
Expansion of B cells in CLL BMMCs

Healthy individual

CLL patient (untreated)

CLL patient (treated but relapsed)

~82% abnormal B cells (CD19+/CD5+) cells detected by immunophenotyping of cell surface markers

82% abnormal B cells (CD19+, CD20+, CD5+, CD10-, CD11c-, CD38-, Lambda+) detected by immunophenotyping of cell surface markers
Distinct Expansions in CLL vs AML BMMCs

Healthy individual

CLL patient (untreated)

AML patient (untreated)

Expansion of B cells (86%)

Expansion of myeloid progenitors (69%)
Additional Early Access Data: Hanlee Ji at Stanford

- Follicular lymphoma: expansion of B cells
- AZD4547 treated KATO III cells