Chromium™
Single Cell 3’
Solution
High-throughput Single Cell Gene Expression

July 2016, Revision B
The Chromium Single Cell 3’ Solution

Chromium™ Controller

• GemCode™ Technology
• Automated
• High-throughput and scalable

Chromium Single Cell 3’ Consumables

• Chip for single cell partitioning in GEMs
• Reagents for RT, amplification and library construction

Cell Ranger™ Pipelines

• Informatics solution for single cell expression profiling
• Pre-processing, QC and analytics
Gel Bead-in-Emulsion (GEM)

- Single T Cell
- Functionalized Gel Bead
- RT Reagents in Solution
- Partitioning Oil
High Diversity Barcode Library

- 750,000 Discrete Reagents in One Tube
- Defined 14 base pair barcode sequence
- Highly uniform size and barcode representation
- Built-in sequencing adapter, barcode and primer
Partitioning and Barcoding of Single Cells

- Super-Poisson loading of barcoded beads into droplets
- Poisson loading of cells in GEMs
- Beads dissolve for efficient, liquid phase biochemistry
- Cell lysis starts immediately following encapsulation
Efficient Scalable Workflow

- Up to 8 channels processed in parallel
- 1,000 to 6,000 cells per channel
- 10 minute run time per chip
- Up to 30 um cell diameter tested
- ~50% cell processing efficiency
- Temperature Range 18-28°C

User controlled trade-off between cell numbers and doublet rate

<table>
<thead>
<tr>
<th>Number of Cells</th>
<th>Expected Doublet Rate (%)*</th>
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</thead>
<tbody>
<tr>
<td>1,200</td>
<td>~1.2</td>
</tr>
<tr>
<td>3,000</td>
<td>~2.9</td>
</tr>
<tr>
<td>6,000</td>
<td>~5.7</td>
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</tbody>
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Assay Scheme for 3’ mRNA Sequencing

In GEM

RT with oligo(dT)VN

First Strand Synthesis

Template Switching RT

mRNA

P7 10X R2 Oligo(dT)VN

P7 10X R2 Oligo(dT)VN

P7 10X R2 Oligo(dT)VN

CCC

GGG

TSO
**Assay Scheme for 3’ mRNA Sequencing**

- **In Bulk**
  - cDNA amplification by PCR
  - Shearing, A tailing, ligation and SI PCR

Diagram:
- Primer
- Oligo(dT)VN
- CCC
- R1
- SI
- P5
Single Cell 3’ End-to-End Workflow

1. Cell preparation
2. Partition and RT inside each GEM
3. Pool and cDNA amplification
4. Covaris shearing
5. Adapter ligation and sample index PCR
6. Sequencing and analysis

Reagents and Consumables in 10X Kit

Total Turn-around Time: ~12 Hrs
Total Hands-on Time: ~4 Hrs
Cell Ranger – Informatics Workflow

• Complete software package for single cell analysis
• Bundled with STAR for efficient transcriptome alignment
• Outputs are standard formats plus Loupe visualization
Cell Ranger – Output Files

**BAM – Genome-Aligned Reads**
- Indexed BAM containing position-sorted, aligned reads
- Barcodes and UMIs attached as standard tags

**MEX – Gene/Barcode Matrix**
- Market Exchange format
- Suitable for downstream analysis in Python and R

**HTML, CSV – Run Summary**
- Run metrics and basic static visualizations

**CSV - Run Analysis**
- 2D projections
- Cell clustering
- Differential expression
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

Combined Expression of Known Phase Markers

• Proliferating HEK293T cells were profiled and scored for expression of markers associated with each major cell cycle phase

• Cells from all phases were identified

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

Whole Blood

- Platelets and Plasma Components
- Mononuclear Cells (PBMCs)
- Erythrocytes (RBCs), Eosinophils, and Neutrophils

Myeloid Cells

- Monocytes
- Macrophages
- Myeloid Dendritic Cells

Lymphoid Cells

- Plasmacytoid Dendritic Cells
- B Cells
- NK Cells
- T Cells
Major populations of PBMCs are detected

- CD45 RA+ Naïve T (26.4%)
- CD4+ T (28.4%)
- CD8+ T (18.7%)
- CD19+ B (5.5%)
- CD14+ Monocytes (5.3%)
- CD34+ Progenitors (0.3%)
- CD56+ NK (13.5%)
- Dendritic (1.9%)
Example: 68,000 Human PBMCs

- CD45RA+Naïve T Cells
- CD4+ T Cells
- CD8+ T Cells
- CD14+ Monocytes
- CD19+ B Cells
- CD34+ Myeloid Progenitors
- CD56+ Natural Killer Cells