TECHNICAL NOTE

Chromium Single Cell 3' v3: Reagent, Workflow & Software Updates

Introduction

The Chromium Single Cell Gene Expression Solution provides a comprehensive, scalable solution for gene expression profiling of hundreds to tens of thousands of cells. The Single Cell 3' v3 reagents and workflow updates provide enhanced sensitivity, enabling detection of even more unique transcripts per cell. This document highlights the key reagent, workflow, and software updates for generating and analyzing Single Cell 3' Gene Expression libraries.

Refer to the Chromium Single Cell 3' Reagent Kits v3 User Guide for the complete protocol.

Single Cell 3' Workflow

VERSION SPECIFIC

The Single Cell 3' v3 workflow (Figure 1) is similar to the Single Cell 3' v2 workflow, with few specific updates that are indicated by a "VERSION SPECIFIC" icon adjacent to the updated protocol steps.

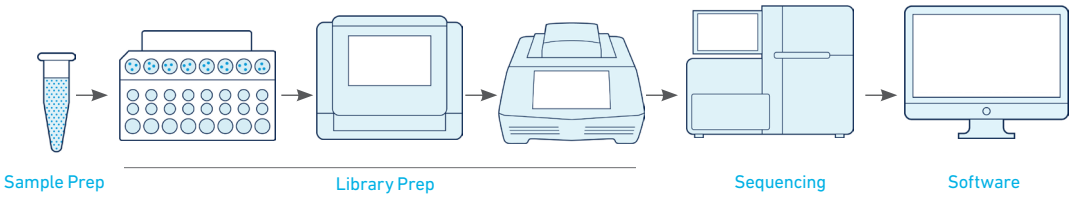


Figure 1. Chromium Single Cell 3' workflow.

Chromium Single Cell 3' v3 Gel Beads

Gel Bead Primers: In addition to the poly(dT) primers that enable the production of barcoded, full-length cDNA from poly-adenylated mRNA, the Single Cell 3' v3 Gel Beads also include two additional primer sequences (Capture Sequence 1 and Capture Sequence 2), that enable capture and priming of Feature Barcoding technology compatible targets or analytes of interest. Only the poly(dT) primers are used for generating Single Cell 3' Gene Expression libraries.

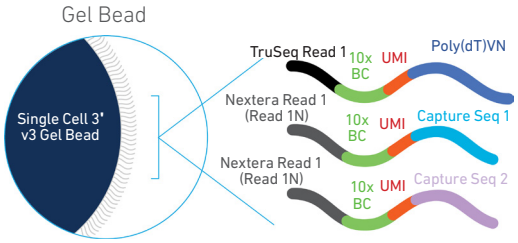


Figure 2. Chromium Single Cell 3' v3 Gel Bead schematic.

Sample Prep

Recommendations for preparing single cell suspensions are unchanged between the Single Cell 3' Reagent Kits v2 and the Single Cell 3' Reagent Kits v3 protocols.
If using Single Cell 3' Reagent Kits v3 protocols with Feature Barcoding technology, additional considerations apply. Visit the 10x Genomics Support website for specifics.

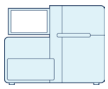
Library Prep

Protocol Steps	Single Cell 3' v2 (Document CG00052)	Single Cell 3' v3 (Document CG000183)
GEM Generation & Barcoding (Reagents cannot be used interchangeably)		
GEM-RT Reagents in Master Mix	RT Reagent Mix RT Enzyme Mix RT Primer* Additive A *Resuspend in 40 µl low TE buffer	RT Reagent RT Enzyme C Template Switch Oligo** Reducing Agent B **Resuspend in 80 µl low TE buffer
Master Mix volume per reaction	66.2 µl	**33.4 µl**
Gel Bead – 10x Barcode Diversity **Gel Bead – UMI Length**	737,000 10 bp	3.5 million 12 bp
Chip Loading (Chips cannot be used interchangeably)		
Gel Beads Master Mix+Cell Suspension Partitioning Oil	Recovery Wells 40 µl 90 µl 270 µl 	Recovery Wells 40 µl*** 75 µl 280 µl  ***Chromium Single Cell 3' v3 Gel Beads
Chromium Controller		
Firmware Version	All versions	**3.16** or higher
Run time	~6.5 min	**~8.5 min**
Post GEM-RT Cleanup & cDNA Amplification		
Dynabeads MyOne SILANE	4 µl	**8 µl**
cDNA Amplification Mix	Nuclease-free Water Amplification Master Mix cDNA Primer Mix cDNA Additive	Amp Mix cDNA Primers† †Includes cDNA Additive
cDNA Amplification – Annealing	Step 3: 67°C	Step 3: **63°C**
cDNA Amplification – Total Cycles	-	Updated recommendations
cDNA Cleanup	0.6X SPRI	0.6X SPRI‡ ‡Different instructions only for Feature Barcoding protocols
Purified cDNA Sample Storage	At -20°C for up to 1 week	At **-20°C** for up to **4 weeks**

Library Prep

Protocol Steps	Single Cell 3' v2 (Document CG00052)	Single Cell 3' v3 (Document CG000183)
3' Gene Expression Library Construction		
Fragmentation Mix	○ Fragmentation Buffer ● Fragmentation Enzyme Blend	● Fragmentation Buffer ● Fragmentation Enzyme
Purified cDNA for Library Construction	40 µl	**10 µl*** *25% of purified cDNA
Adaptor Ligation Mix	Nuclease-free Water ● Ligation Buffer ● DNA Ligase ● Adaptor Mix	● Ligation Buffer ● DNA Ligase ● Adaptor Oligos

Sequencing



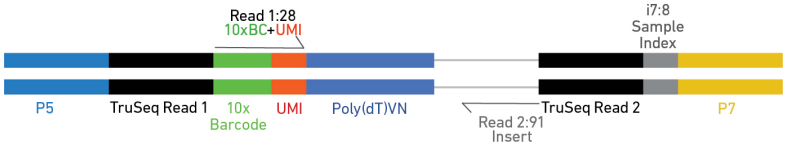


Figure 3. Chromium Single Cell 3' Gene Expression v3 Library.

Sequencing Read	Single Cell 3' Gene Expression v2**	Single Cell 3' Gene Expression v3
Read1 i7 Index Read2	26 cycles 8 cycles 98 cycles	28 cycles 8 cycles 91 cycles
Sequencing Depth	50,000 read pairs per cell	Minimum 20,000 read pairs per cell

**Single Cell 3' Gene Expression v2 libraries may be sequenced using the configuration for Single Cell 3' Gene Expression v3 libraries. However, v3 libraries should not be sequenced using the v2 configuration as 28 Read 1 cycles are required to capture the 16 nt 10x Barcode and the 12 nt UMI sequences.

Software

Cell Ranger 3.0 and Loupe Cell Browser 3.0 are required for analyzing and visualizing data generated from Single Cell 3' v3 libraries. The updated software includes increased sensitivity of cell calling for low-RNA-content cell types, offers optional chemistry batch effect correction to aggregate Single Cell 3' v2 and Single Cell 3' v3 chemistry data, and filters chimeric reads that add technical noise at high depths.

Cell Ranger 3.0 has been updated to support user-defined Feature Barcoding reagents, and to quantify these features alongside standard gene expression libraries. Cell Ranger 3.0 also includes updates to some pipeline algorithms and outputs.

Loupe Cell Browser 3.0 has been updated to allow users to easily analyze data generated with the Feature Barcoding technology for Gene Expression and Immune Profiling applications, as well as from the new Chromium Single Cell ATAC solution.
For more information about the updates, please visit the 10x Genomics Support website

Version Compatibility
Cell Ranger 3.0 is backward compatible and can be used to analyze Single Cell 3' Gene Expression v1, v2 and v3 chemistry data. Loupe Cell Browser is also backward compatible and can be used to visualize .cloupe files generated by Cell Ranger versions 1.3 and higher.

Chromium Single Cell 3’ v3 – Product List & Documents

Product list for generating Chromium Single Cell 3' Gene Expression Libraries:

REAGENT KITS	REACTIONS	PART NUMBER (PN)
Chromium Single Cell 3' GEM, Library & Gel Bead Kit v3	16 rxns 4 rxns	1000075 1000092
Chromium Chip B Single Cell Kit Chromium Chip B Single Cell Kit	48 rxns 16 rxns	1000073 1000074
Chromium i7 Multiplex Kit	96 rxns	120262
INSTRUMENT		
Chromium Single Cell Controller & Accessory Kit		120223
Chromium Controller & Accessory Kit		120263
SOFTWARE		
Cell Ranger Analysis Pipeline (DOWNLOAD)		
Loupe Cell Browser (DOWNLOAD)		
DOCUMENTS (for Single Cell 3' v3 Gene Expression Libraries ONLY)		
User Guide : Chromium Single Cell 3' Reagent Kits v3 (CG000183)		

If using Chromium Single Cell 3' Reagent Kits v3 protocols with Feature Barcoding technology, the Chromium Single Cell 3' Feature Barcode Library Kit is required in addition to all the products listed above. Refer to the indicated documents for specific guidance.

REAGENT KITS	REACTIONS	PART NUMBER (PN)
Chromium Single Cell 3' Feature Barcode Library Kit	16 rxns	1000079
DOCUMENTS (for Single Cell 3' v3 Gene Expression + Single Cell 3' CRISPR Screening Libraries ONLY)		
User Guide : Chromium Single Cell 3' Reagent Kits v3 with Feature Barcoding technology for CRISPR Screening (CG000184) Tech Note : Guide RNA Specifications Compatible with Feature Barcoding Technology for CRISPR Screening (CG000197)		
DOCUMENTS (for Single Cell 3' v3 Gene Expression + Single Cell 3' Cell Surface Protein Libraries ONLY)		
User Guide : Chromium Single Cell 3' Reagent Kits v3 with Feature Barcoding technology for Cell Surface Protein (CG000185) Demonstrated Protocol : Cell Surface Protein Labeling for Single Cell RNA Sequencing Protocols (CG000149)		

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