TECHNICAL NOTE

# Chromium Next GEM Single Cell 3' v3.1: Reagent & Workflow 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. This document highlights the key changes in Chromium Next GEM Single Cell 3' v3.1 reagents, along with workflow updates for generating and analyzing Illumina-ready sequencing libraries.

Refer to the Chromium Next GEM Single Cell 3' Reagent Kits v3.1 User Guides for the complete protocol.

## Chromium Next GEM Single Cell 3' v3.1 Workflow

Next GEM

The Chromium Next GEM Single Cell 3' v3.1 workflow (Figure 1) is similar to the Single Cell 3' v3 workflow, with a few specific updates that are indicated by a "Next GEM" icon adjacent to the updated steps in the Chromium Next GEM Single Cell 3' Reagent Kits v3.1 User Guides .

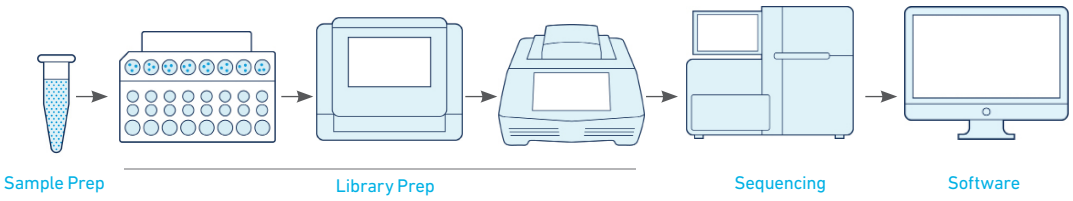


**Figure 1**. Chromium Next GEM Single Cell 3' v3.1 workflow.

## Chromium Next GEM Chip G

The Chromium Next GEM Chip G is assembled in the Chromium Next GEM Secondary Holder. The location of the rows labeled 1-3 and the NO FILL (not used) wells are unique to the Chromium Next GEM Chip G (Figure 2A). GEMs are generated by combining a Master Mix containing cells and enzymes, barcoded Single Cell 3' v3.1 Gel Beads, and Partitioning Oil onto the Chromium Next GEM Chip G (Figure 2B).

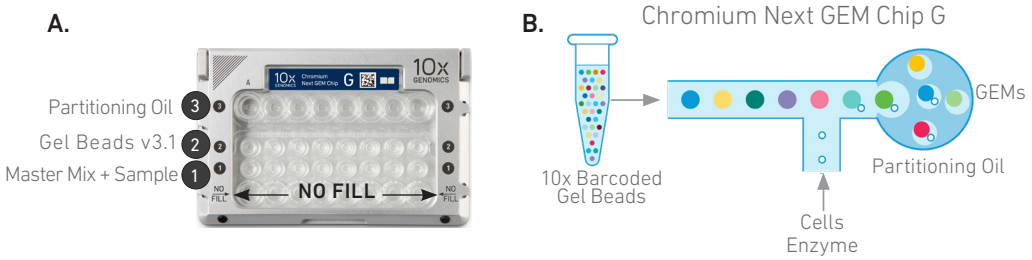


**Figure 2**. Chromium Next GEM Chip G assembled in a Chromium Next GEM Secondary Holder (A). GEM generation in Chromium Next GEM Chip G (B).

Next GEM reagents are specific to Next GEM products and should not be used interchangeably with non-Next GEM reagents.

## Sample Prep

Recommendations for preparing single cell suspensions are unchanged for Chromium Next GEM Single Cell 3' Reagent Kits v3.1 protocols.

## Library Prep

| Protocol Steps | Single Cell 3' v3 | Next GEM Single Cell 3' v3.1 |
|---|---|---|
| **Reagent Kits** | | |
| **10x Genomics Reagents** | Chromium Single Cell 3' Reagent Kits v3 | Chromium Next GEM Single Cell 3' Reagent Kits v3.1 |
| | Chromium Single Cell 3' Feature Barcode Library Kit | Chromium Single Cell 3' Feature Barcode Library Kit |
| | Chromium i7 Multiplex Kit | Chromium i7 Multiplex Kit |
| **GEM Generation & Barcoding** | | |
| **GEM-RT Reagents in Master Mix** | RT Reagent | RT Reagent B |
| **Master Mix volume** | 33.4 µl | **31.8 µl** (updated reagent volumes in Master Mix) |
| **Cell Suspension Volume Table** | - | Updated volumes |
| **Gel Bead** | Single Cell 3' v3 Gel Beads | Single Cell 3' v3.1 Gel Beads (updated handling instructions; vortex 30 sec, centrifuge 5 sec before use) |
| **Chip Loading** (Chips cannot be used interchangeably) | | |
| |   |   |
| **Master Mix + Sample Gel Beads Partitioning Oil** | 75 µl - row labeled 1<br>40 µl - row labeled 2<br>280 µl - row labeled 3 | **70 µl - row labeled 1**<br>**50 µl - row labeled 2***<br>**45 µl - row labeled 3****<br>* After loading the Gel Beads, wait 30 sec before loading Partitioning Oil.<br>**After loading Partitioning Oil, attach the gasket and run the chip in the Chromium Controller **immediately** |
| **Chromium Controller** | | |
| **Firmware Version** | 3.16 or higher | **4.0 or higher** |
| **Run time** | ~8.5 min | **~18 min** |
| **GEM Recovery** | | |
| | From top row labeled "NO FILL" | From top row labeled "3" |
| | NO FILL  |   |

## Sequencing

Recommendations for sequencing libraries generated using the Chromium Next GEM Single Cell 3' Reagent Kits v3.1 protocols are same as sequencing recommendations for libraries generated using the Chromium Single Cell 3' Reagent Kits v3 protocols.

## Software

Libraries generated using the Chromium Next GEM Single Cell 3' Reagent Kits v3.1 can be analyzed and visualized using the current versions of Cell Ranger and Loupe Cell Browser.

# Chromium Next GEM Single Cell 3' v3.1 – Product List & Documents

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

| REAGENT KITS | REACTIONS | PART NUMBER (PN) |
|---|---|---|
| Chromium Next GEM Single Cell 3' GEM, Library & Gel Bead Kit v3.1 | 16 rxns<br>4 rxns | 1000121<br>1000128 |
| Chromium Next GEM Chip G Single Cell Kit | 48 rxns<br>16 rxns | 1000120<br>1000127 |
| Chromium Next GEM Single Cell 3' Library Construction Kit v3.1 (for addititional libraries) | 16 rxns | 1000157 |
| Chromium i7 Multiplex Kit | 96 rxns | 120262 |
| **INSTRUMENT** | | |
| Chromium Controller & Next GEM Accessory Kit | - | 120223 (12 month warranty)<br>120246 (24 month warranty) |
| **SOFTWARE** | | |
| Cell Ranger Analysis Pipeline (DOWNLOAD) | | |
| Loupe Cell Browser (DOWNLOAD) | | |
| **DOCUMENTS** (for Single Cell 3' Gene Expression Libraries ONLY) | | |
| User Guide : Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (CG000204) | | |

If using Next GEM Single Cell 3' Reagent Kits v3.1 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' Gene Expression + Single Cell 3' CRISPR Screening Libraries ONLY) | | |
| User Guide : Chromium Next GEM Single Cell 3' Reagent Kits v3.1 with Feature Barcoding technology for CRISPR Screening (CG000205)<br>Tech Note : Guide RNA Specifications Compatible with Feature Barcoding Technology for CRISPR Screening (CG000197) | | |
| **DOCUMENTS** (for Single Cell 3' Gene Expression + Single Cell 3' Cell Surface Protein Libraries ONLY) | | |
| User Guide : Chromium Next GEM Single Cell 3' Reagent Kits v3.1 with Feature Barcoding technology for Cell Surface Protein (CG000206)<br>Demonstrated Protocol : Cell Surface Protein Labeling for Single Cell RNA Sequencing Protocols (CG000149) | | |



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