NEXT GEM

CHROMIUM SYSTEM | APPLICATION NOTE
SINGLE CELL ASSAY FOR TRANSPOSASE ACCESSIBLE CHROMATIN

Performance of Chromium Next GEM Single Cell ATAC Reagent Kits

SUMMARY

The Chromium Single Cell ATAC (Assay for Transposase Accessible Chromatin) Solution provides a robust and scalable approach to profile the chromatin landscape of single cells. Here, we introduce the next generation of 10x Genomics' microfluidic chip technology and reagents (Chromium Next GEM) for the Chromium Single Cell ATAC Solution. We demonstrate comparable performance between the Chromium Next GEM Single Cell ATAC v1.1 with Chromium Single Cell ATAC (v1.0) by assessing cell clustering and key metrics including median unique fragments per cell, multiplet rate, and cell recovery on various sample types.

HIGHLIGHTS

- Chromium Next GEM Single Cell ATAC Reagent Kit v1.1 has comparable performance to Chromium Single Cell ATAC Reagent Kit v1.0
- Minimal to no batch effect between versions
- Chromium Next GEM is built on the new chip architecture, enabling future solutions and product improvements

INTRODUCTION

Chromatin accessibility is an important tool for understanding epigenetic regulation of the genome. Chromatin compaction and DNA binding proteins provide a regulatory layer in addition to DNA sequence that influences gene expression. Assaying chromatin landscapes at the single cell level can inform distinct cell types and states, while providing insight into gene regulatory networks. To profile chromatin accessibility in single cells, we have enabled the next generation of microfluidic chip architecture and reagents (Chromium Next GEM) as a part of our Chromium Single Cell ATAC Solution. The Chromium Next GEM workflow is outlined in Figure 1.

The Chromium Next GEM Single Cell ATAC Workflow

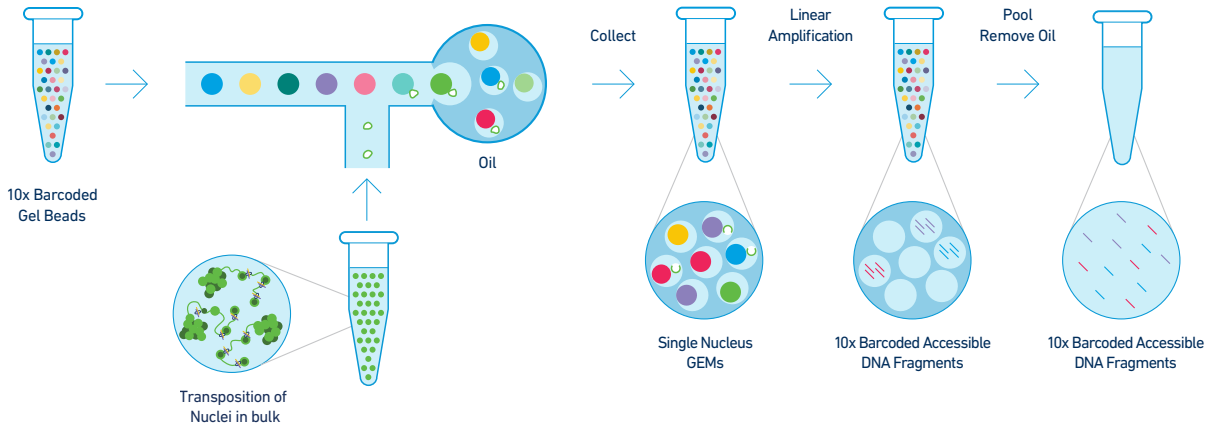

Figure 1. The Chromium Next GEM Single Cell ATAC workflow. Nuclei are transposed in bulk, followed by partitioning on a microfluidic chip into nanoliter-scale GEMs. The transposed DNA of individual nuclei are identified with a unique 10x barcode. Libraries are generated and sequenced, and 10x barcodes are used to associate individual reads back to individual partitions and, thereby, each individual cell.

10x GENOMICS

METHODS

Single Cell ATAC libraries were generated from (1) unstimulated peripheral blood mononuclear cells (PBMCs; AllCells) and (2) 1:1 mixture of a human B-lymphocyte cell line, GM12878 (Coriell Institute), and a mouse B-lymphocyte cell line, A20 (ATCC), for the mixed species experiments. The Demonstrated Protocol for Nuclei Isolation for Single Cell ATAC Sequencing (Document CG000169) was followed to isolate nuclei.

Libraries were constructed with both the Next GEM Single Cell ATAC v1.1 and Single Cell ATAC reagent and chip kits following the procedure in their respective User Guides:

- Chromium Single Cell ATAC Library & Gel Bead Kit, and Chromium Chip E Single Cell ATAC Kit (Document CG000168)
- Chromium Next GEM Single Cell ATAC Library & Gel Bead Kit v1.1, and Chromium Next GEM Chip H Single Cell ATAC Kit (Document CG000209)

Libraries were sequenced on a NovaSeq 6000 to approximately ~38,000 raw read pairs per cell using the following read configuration: Read 1 50 cycles , i7 read 8 cycles, i5 read 16 cycles, Read 2 49 cycles. The data from both reagent chemistries were analyzed using the Cell Ranger ATAC pipeline v1.1.0.

RESULTS

Single Cell ATAC libraries were generated from ~5,000, or ~10,000 PBMCs using either Single Cell ATAC Reagent Kits (hereafter referred to as "v1.0") or Next GEM Single Cell ATAC v1.1 Reagent Kits. Data was analyzed with Cell Ranger ATAC (v1.1.0). Clusters were assigned cell identities using differential chromatin peaks identified from sorted FACS populations (Figure 2A and B). Both libraries show similar distinct clusters and cell identities (monocytes, B cells, CD4+ T cells, CD8+ T cells, natural killer (NK) cells, and dendritic cells).

Comparable Clustering of PBMCs using Single Cell ATAC v1.0 and Next GEM Single Cell v1.1

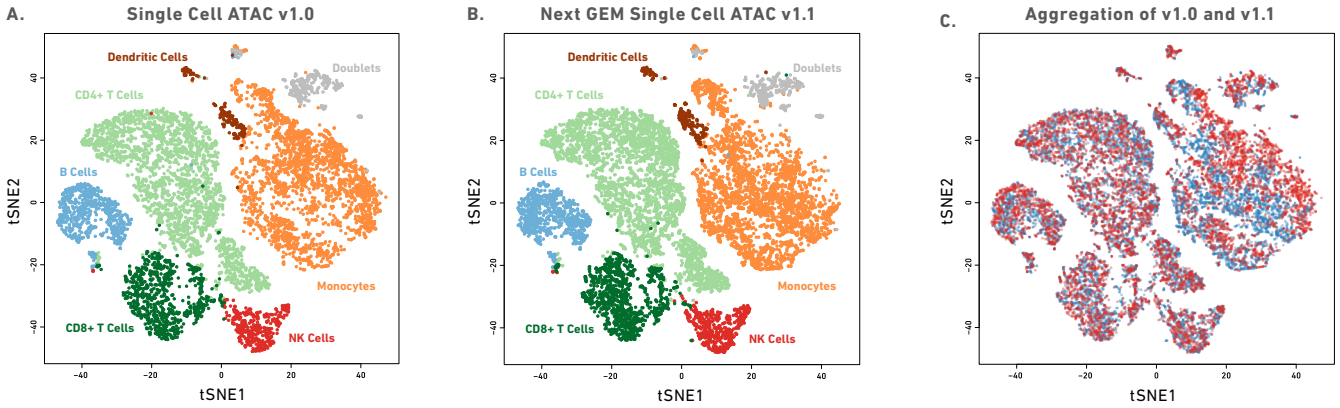

Figure 2. Single Cell ATAC libraries generated from both library chemistries show comparable clustering and relative abundance of all major cell types. (a) tSNE plot generated from 8,161 PBMCs using Chromium Single Cell ATAC Reagent Kits (v1.0). (b) tSNE plot generated from 10,247 PBMCs using Chromium Next GEM Single Cell ATAC Reagent Kits (v1.1). (c) Aggregated analysis indicates minimal technical differences between the two reagent versions (Red, v1.0, Blue, v1.1).

To directly compare the data generated with v1.0 and v1.1, PBMC libraries were aggregated and analyzed using Cell Ranger ATAC pipeline (cellranger-atac aggr; default settings). The aggregated clustering plot is shown in Figure 2C. The libraries feature largely overlapping populations of cells, demonstrating comparable performance. In addition, the proportion of cell types detected is similar in each library and across different cell loading tests (Figure 3).

Proportion of Cell Types Detected in PBMC Samples Run with Single Cell ATAC v1.0 and Next GEM Single Cell ATAC v1.1

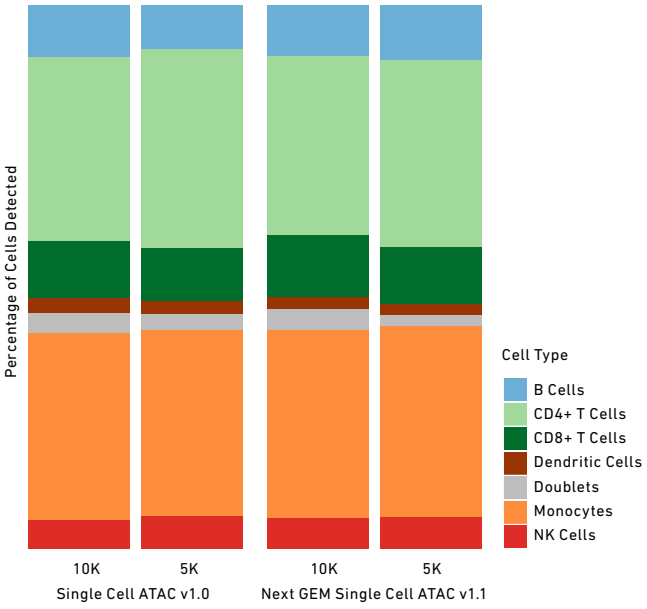

Figure 3. The proportion of cell types detected with the Chromium Single Cell ATAC Reagents Kit (v1.0) and Chromium Next GEM Single Cell ATAC Reagents Kit (v1.1) are similar and consistent across technical replicates. Two different libraries, targeting 5,000 and 10,000 cells, are shown for each.

To assess multiplet rate between v1.0 and v1.1, a mixed-species experiment was performed using a 1:1 mixture of human and mouse lymphocytes. Libraries were generated using both v1.0 and v1.1, and ~1000 nuclei were targeted. Figure 4 shows comparable levels of multiplets (<2% for 1,000 targeted nuclei) across the two chips (multiplet rate for v1.0: 1.7%; multiplet rate for v1.1: 0.8%).

Low Multiplet Rates Observed for Samples Run on Single Cell ATAC v1.0 and Next GEM Single Cell ATAC v1.1

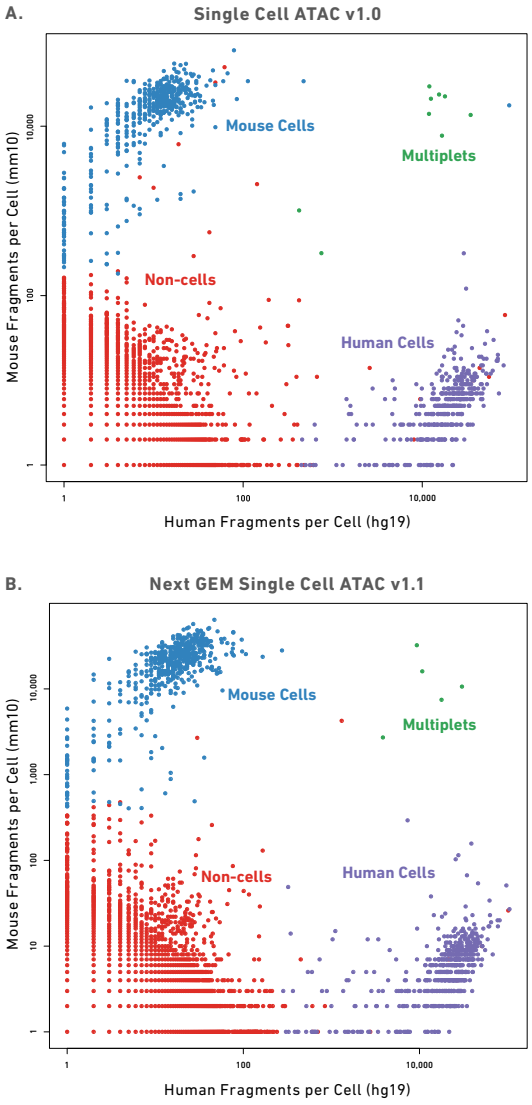

Figure 4. Number of fragments mapping to the human or mouse genome for each 10x barcode. 10x barcode not detected as cells are red. Cells with fragments mapping to human (purple), mouse (blue), or both (green, multiplets) are shown for (a) Single Cell ATAC Reagents Kit v1.0 (1.7%, 1,051 total nuclei barcodes detected) and (b) Next GEM Single Cell ATAC Reagents Kit v1.1 (0.8%, 1,246 total nuclei barcodes detected). The multiplet rate is low (<2%) for both versions.

Sample Type	Reagent Kit	Cells Loaded	Cells Detected	Median Unique Fragments per Cell (hg19)		Fragments overlapping Peaks
				15K	25K	%
PBMC	v1.0	15,300	8,161	6,362	8,930	67
	Next GEM v1.1	15,300	10,247	6,535	9,446	69
Human:Mouse	v1.0	1,530	1,051	10,117	15,325	55
	Next GEM v1.1	1,530	1,246	9,945	15,114	55

Table 1. Library complexity and enrichment in Single Cell ATAC and Next GEM Single Cell ATAC libraries across sample types. The Median Unique Fragments per Cell is provided at different sequencing depths (15K, 15,000 read pairs per cell; 25K, 25,000 read pairs per cell). For mixed-species experiments, this metric is reported for reads aligning to the human reference (hg19). 'Fragments overlapping peaks' is the percentage of aligned reads that overlap the peak set called by Cell Ranger ATAC.

To compare library complexity between v1.0 and v1.1, sequencing reads from PBMC and mixed-species libraries were computationally downsampled to 15,000 or 25,000 raw read pairs per cell and the number of median unique fragments per cell was tabulated (Table 1). Similar library complexity was observed for both reagent kits.

CONCLUSIONS

In this Application Note, we describe the performance of Chromium Next GEM Single Cell ATAC Reagent Kits v1.1 compared to Chromium Single Cell ATAC Reagent Kits (v1.0). By assessing Single Cell ATAC libraries generated from various sample types, we demonstrate that both product versions yield comparable results in terms of clustering, the proportion of subpopulations detected, and library complexity. In addition, the recovery efficiency of nuclei and the multiplet rate are not affected. Importantly, we observed minimal batch effect when comparing Single Cell ATAC libraries from biological samples generated on the two reagent kit versions. These data highlight comparable performance of Next GEM technology.

RESOURCES

Datasets	go.10xgenomics.com/scATAC/datasets
Seminars	go.10xgenomics.com/scATAC/seminars
Application Notes	go.10xgenomics.com/scATAC/app-notes
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