Unify single-cell gene expression and chromatin accessibility

- Leverage Chromium Single Cell Multiome ATAC + Gene Expression from 10x Genomics for multiomic measurements from the same single cells

- Sequence paired ATAC-Seq and 3' gene expression libraries on the NovaSeq™ X, NovaSeq 6000, NextSeq™ 2000, NextSeq 1000, or NextSeq 550 System

- Connect single-cell transcriptome and epigenome results precisely for a holistic view of gene regulation

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Introduction

Single-cell analysis can help tease apart heterogeneity in complex cell populations, distinguishing cell types and revealing dynamic cell states. Next-generation sequencing (NGS) enables innovative assays to examine the genome, transcriptome, epigenome, or proteome with single-cell resolution for hundreds to tens of thousands of cells. Researchers can integrate these complementary metrics into multiomic data sets to gain a deeper understanding of cellular phenotypes.

Many multiomic approaches rely on sophisticated algorithms and inferences about correlation across modalities to connect data from separate experiments.[1] However, single-cell sequencing workflows build multiomics directly into the assay by interrogating multiple cellular features in the same cell and linking results using oligonucleotide barcodes.

This technical note outlines a protocol for simultaneous profiling of the transcriptome (using 3' gene expression) and epigenome (using ATAC-Seq; assay for transposase-accessible chromatin with sequencing) from single cells. Precisely connecting single-cell gene expression and chromatin accessibility from the same cell can illuminate how genes are expressed and regulated across different cell types.

Protocol overview

This single-cell multiomics experiment follows a straightforward workflow of library preparation, sequencing, and analysis (Figure 1). The protocol leverages Chromium Next GEM Single Cell Multiome ATAC + Gene Expression from 10x Genomics and proven sequencing power from Illumina. Beginning with a nuclei suspension, use the Chromium X/iX and reagents to generate two barcoded sequencing-ready "multiome" libraries: one single-cell ATAC-Seq library and one single-cell gene expression library.[2] Sequence the paired multiome libraries on an Illumina production-scale sequencing system, such as the NovaSeq X, NovaSeq 6000, NextSeq 2000, NextSeq 1000, or NextSeq 550 System. Data analysis with the Cell Ranger ARC pipeline or Illumina DRAGEN™ Single-Cell app links ATAC-Seq results and gene expression results for any individual cell using their cellular barcode. Loupe Browser software makes it easy to visualize and explore single-cell multiomic data.

Prepare samples

Sample preparation starts with isolating a nuclei suspension from cell culture, primary cells, or fresh or frozen tissue. The assay has been validated on various cell lines, peripheral blood mononuclear cells (PBMCs), fresh and frozen embryonic mouse brain, frozen human brain, and frozen lymph node tumor, and also can be applied to other sample types.[3]

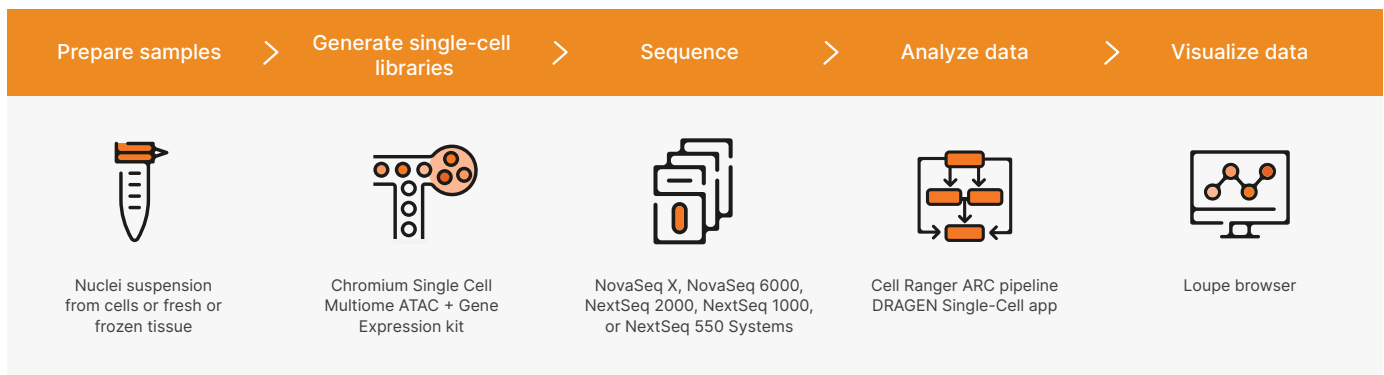| Prepare samples	>	Generate single-cell libraries	>	Sequence	>	Analyze data	>	Visualize data
Nuclei suspension from cells or fresh or frozen tissue		Chromium Single Cell Multiome ATAC + Gene Expression kit		NovaSeq X, NovaSeq 6000, NextSeq 2000, NextSeq 1000, or NextSeq 550 Systems		Cell Ranger ARC pipeline DRAGEN Single-Cell app		Loupe browser

Figure 1: Workflow for a single-cell multiomic experiment—Start with a nuclei suspension, followed by microfluidic single-cell partitioning and barcoding with the Chromium X/iX. Sequence the two resulting multiome libraries (one single-cell ATAC-Seq library and one single-cell gene expression library) on Illumina systems. Analyze data using Cell Ranger ARC or the Illumina DRAGEN Single-Cell app (available on BaseSpace™ Sequence Hub or locally onboard the NextSeq 1000 and NextSeq 2000 Systems). Visualize data with Loupe Browser software.

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High-quality nuclei are critical for good results. Demonstrated protocols for nuclei isolation from various sample types are available on the 10x Genomics Support website.[4] These demonstrated protocols contain guidelines for freezing cell suspensions and tissues, cleanup methods such as DNase treatment, removal of granulocyte cells via fluorescence-activated cell sorting (FACS), and sorting of nuclei via FACS.[3]

Generate single-cell libraries

Once nuclei are isolated, they are ready for library prep using the Chromium Next GEM Single Cell Multiome ATAC + Gene Expression kit (Figure 2).[4] Nuclei are treated in bulk with a hyperactive transposase enzyme to cut and insert sequencing adapters into exposed DNA.[5] Transposed nuclei are loaded onto a microfluidic chip and run in the Chromium X/iX (10x Genomics), which partitions individual nuclei into droplets, each with a single gel bead that contains a unique barcode. The droplets, or "GEMs" (Gel bead-in-emulsion), are then incubated to attach barcodes to both mRNA and transposed DNA fragments from the same nuclei. This step links ATAC-Seq results and gene expression results.

Following this incubation, GEMs are broken and pooled fractions are recovered and purified. These fractions are taken through a pre-amplification polymerase chain reaction (PCR) step to fill gaps and ensure maximum recovery of barcoded transposed DNA and cDNA fragments.

The pre-amplified product is then used as input for both ATAC-Seq library construction and cDNA amplification for gene expression library construction.[3] The resulting barcoded single-cell multiome gene expression and ATAC-Seq libraries are now ready for sequencing using Illumina NGS sequencing systems.

Sequence with Illumina instruments

To handle the sequencing output required for this application, we recommend sequencing the paired multiome libraries on the NovaSeq X, NovaSeq 6000, NextSeq 2000, NextSeq 1000, or NextSeq 550 Systems (Table 1). Read configuration is dependent on the library type (Table 2, Table 3).

For multiome ATAC-Seq libraries the PhiX spike-in recommendation is 1%. The spike-in helps ensure appropriate sequencing diversity to assist in high-quality sequencing.[7] Expected sequencing metrics for the single-cell multiome gene expression library[8] and the single-cell multiome ATAC-Seq library[9] are available on the 10x Genomics Support website.

For more information visit Single Cell Multiome ATAC + Gene Expression
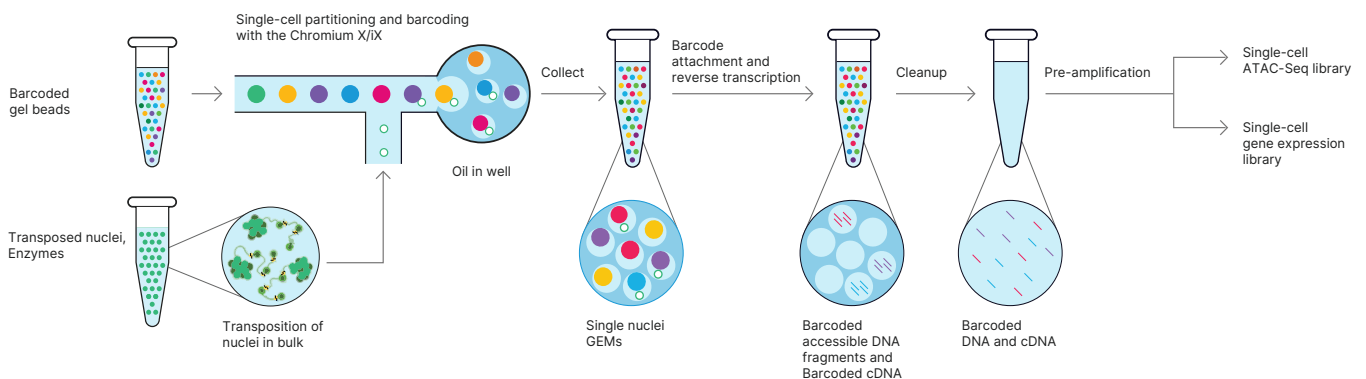

Figure 2: Generate 3' gene expression and ATAC-Seq libraries from the same cells—Starting with a single nuclei suspension, transposition is performed in bulk before individual nuclei are captured in GEMs, where DNA fragments and the 3' ends of mRNA are barcoded. GEMs are broken and pooled before cleanup, pre-amplification, and library construction. This generates two complementary libraries from each sample to link gene expression and open chromatin profiles back to the same cell with certainty.[2]

Table 1: Example sample throughput for Chromium Single Cell Multiome assay on Illumina sequencing systems

Library type	Minimum read pairs per nucleus[a]	Nuclei per sample	No. of samples per run									
			NextSeq 2000[d]		NovaSeq 6000				NovaSeq X		
			P2[e]	P3	SP	S1	S2	S4	1.5B	10B	25B
Multiome 3' gene expression	20K[b]	5K	4	11	8	16	40	100[f]	15	100	250
Multiome ATAC-Seq	25K[b,c]	5K	4	8	6	12	32	80	12	80	200

a. Minimum read recommendations courtesy of 10x Genomics.[7]
b. Adjust sequencing depth for the required performance or application. The sequencing saturation metric and curve in the Cell Ranger ARC run summary can be used to optimize sequencing depth for specific sample types.
c. 50K individual reads; 25K from Read 1 and 25K from Read 2.
d. 4 samples per run for Multiome 3' gene expression; 3 samples per run for Multiome ATAC-Seq libraries on the NextSeq 550 System.
e. P2 flow cells with the same sample throughput also available on the NextSeq 1000 System.
f. Individual lane loading required for loading 100 samples.

Table 2: Recommended read configuration for Chromium Single Cell Multiome libraries

	Multiome 3' gene expression libraries				Multiome ATAC-Seq libraries			
	Read 1	i7 index	i5 index	Read 2	Read 1	i7 index	i5 index	Read 2
Purpose	Cell barcode and UMI	Sample index	Sample index	cDNA insert	Transposed DNA	Sample index	Cell barcode	Transposed DNA
Length[a]	28 bp	10 bp	10 bp	90 bp	50 bp[b]	8 bp	24 bp[c]	49 bp[b]

a. Shorter transcript reads may lead to reduced transcriptome alignment rates. Cell barcode, unique molecular identifier (UMI), and sample index reads must not be shorter than indicated. Any read can be longer than recommended. Cell Ranger ARC will automatically ignore any additional bases in cell barcode or UMI reads.
b. Sequencing length can be adjusted based on the sequencing kit used, but should not be less than 30 bp.
c. Sequencing systems that do not support a 24-bp i5 index read, such as the NextSeq 550 System, require a custom recipe.[10]

Table 3: Analysis options for Chromium Single Cell Multiome assay on Illumina sequencing systems

System	Custom recipe required	FASTQ generation options	Multiome analysis options
NextSeq 500 and NextSeq 550 Systems	Yes (Multiome ATAC-Seq libraries) No (Multiome 3' gene expression libraries)	Onboard instrument bcl2fastq bcl-convert Cell Ranger ARC mkfastq	Cell Ranger ARC DRAGEN Single-Cell app
NextSeq 1000 and NextSeq 2000 Systems	No	Onboard instrument bcl2fastq bcl-convert Cell Ranger ARC mkfastq	Cell Ranger ARC DRAGEN Single-Cell app
NovaSeq 6000 System	No	bcl2fastq bcl-convert Cell Ranger ARC mkfastq	Cell Ranger ARC DRAGEN Single-Cell app
NovaSeq X Series[a]	No	bcl2fastq bcl-convert	Cell Ranger ARC DRAGEN Single-Cell app

a. Sequencing data generated on the NovaSeq X Series are converted to FASTQ files using Illumina demultiplexing pipelines (bcl2fastq or bcl-convert).

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Analyze and visualize your data

Following sequencing, the Cell Ranger ARC analysis pipeline (10x Genomics) identifies open chromatin regions and simultaneously counts transcripts and peak accessibility in single cells. Since the ATAC-Seq and transcriptome measurements are on the same cell, you are able to directly link the readouts of chromatin accessibility and gene expression.

Cell Ranger ARC software also identifies clusters of cells with similar profiles. The analysis pipeline output includes QC information[11] and files that you can use for further analysis in Loupe Browser visualization software (10x Genomics) or third-party R or Python tools.

Illumina DRAGEN Single-Cell app, available either in the cloud, on premises server, or onboard the NextSeq 1000 and NextSeq 2000 Systems, also offers tools to analyze single-cell multiomics data. This powerful analysis pipeline outputs quality control metrics and a cell-by-gene expression matrix that is compatible with popular single-cell analysis tools, including Scanpy, AnnData, and Seurat.

Data highlights

Linked transcriptomic and epigenomic analyses enable deep characterization of cell types and states. For example, by comparing transcription factor expression with motif accessibility in the same cell types, you can identify drivers of differential gene expression and refine regulatory networks (Figure 3).[2] You can also use Loupe Browser to visualize linkages between open chromatin peaks that are associated with nearby gene expression (Figure 4).[2]

Access expert support

Illumina and 10x Genomics technical support teams are available to support you throughout the Chromium Single Cell Multiome ATAC + Gene Expression workflow. Contact 10x Genomics Support (support@10xgenomics.com) for assay and analysis questions, and Illumina Support (techsupport@illumina.com) for sequencing questions. The teams are also equipped to handle more complex issues together.

A. PBMCs gene expression projection

- B cells
- CD14 monocytes
- CD16 monocytes
- myeloid DC
- plasmacytoid DC
- naïve T cells
- memory CD4 T cells
- memory CD8 T cells
- CD16(bright) NK cells
- CD16(dim) NK cells

B. *NFE2L2* gene expression

logNormalized counts
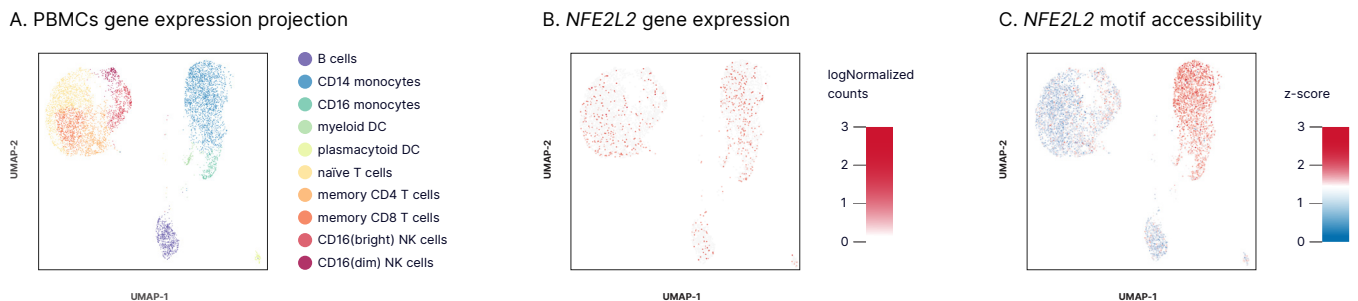
C. *NFE2L2* motif accessibility

z-score

Figure 3: Complementary single-cell data for gene expression and chromatin state—Nuclei extracted from healthy PBMCs were processed using Chromium Single Cell Multiome ATAC + Gene Expression and libraries were sequenced on the NovaSeq 6000 System. (A) Cluster analysis was performed on 7273 nuclei using gene expression data; cell populations annotated based on established marker genes. (B) Expression of the transcription factor *NFE2L2* is observed across cell types. (C) However, *NFE2L2* motif accessibility derived from ATAC-Seq data from the same cells is restricted to monocyte populations.[2]
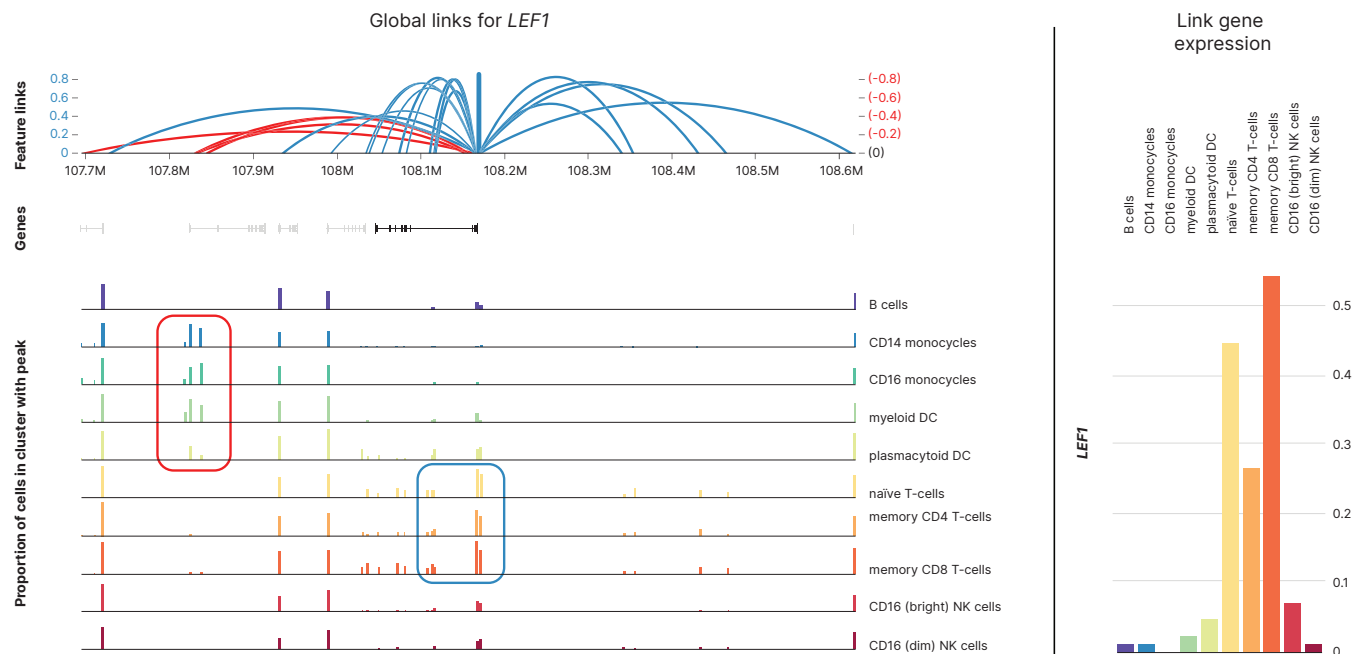
Figure 4: Identification of putative regulatory elements directly linked to a gene of interest—Global links for *LEF1* indicate open chromatin peaks that are either correlated (blue arcs) or anti-correlated (red arcs) with *LEF1* gene expression across a 1 Mb window for the same 7273 PBMC nuclei seen in Figure 3. *LEF1* expression levels and open chromatin peaks are color coded by cell type. Cell-type specific expression of *LEF1* is correlated with linked open chromatin regions near the *LEF1* promoter that are enriched specifically in naïve and memory T-cells (blue box). Cells with low *LEF1* expression, such as monocytes and myeloid dendritic cells, each have an open chromatin region several hundred kilobases away that may be repressive (red box).[2]

Summary

This single-cell multiomics protocol enables simultaneous profiling of gene expression and chromatin accessibility from single cells. The unified data set can help reveal cellular mechanisms driving gene regulation, including gene expression differences in healthy and disease states.

Learn more

[Single-cell sequencing](#)

[Chromium Single Cell Multiome ATAC + Gene Expression](#)

[High-accuracy next-generation sequencing with the NovaSeq X Series](#)

References

1. Stuart T, Butler A, Hoffman P, et al. Comprehensive Integration of Single-Cell Data. *Cell*. 2019;177(7):1888-1902.e21. doi:10.1016/j.cell.2019.05.031
2. 10x Genomics. Simultaneous profiling of the transcriptome and epigenome from the same cell. Accessed October 25, 2023.
3. 10x Genomics. Single Cell Multiome ATAC + Gene Expression Support. support.10xgenomics.com/single-cell-multiome-atac-gex. Accessed October 25, 2023.
4. 10x Genomics. Single Cell Multiome ATAC + Gene Expression Sample Prep Demonstrated Protocols. support.10xgenomics.com/single-cell-multiome-atac-gex/sample-prep. Accessed October 25, 2023.
5. Buenrostro J, Wu B, Chang H, Greenleaf W. ATAC-seq: a method for assaying chromatin accessibility genome-wide. *Curr Protoc Mol Biol*. 2015;109:21.29.1-21.29-9. doi:10.1002/0471142727.mb2129s109
6. 10x Genomics. Specifying Input FASTQ Files for cellranger-arc count. Accessed October 10, 2023.
7. 10x Genomics. Sequencing Requirements for Single Cell Multiome ATAC + Gene Expression. Accessed October 10, 2023.
8. 10x Genomics. Sequencing Metrics & Base Composition of Single Cell 3' v3.1 Dual Index Libraries. Accessed October 25, 2023.
9. 10x Genomics. Sequencing Metrics & Base Composition of Single Cell Multiome ATAC Libraries. Accessed October 10, 2023.
10. 10x Genomics. Why do I need a custom recipe when sequencing Multiome ATAC libraries on the NextSeq? Accessed October 25, 2021.
11. 10x Genomics. Interpreting Cell Ranger ARC Web Summary Files for Single Cell Multiome ATAC + Gene Expression Assay. Accessed October 25, 2023.

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1.800.809.4566 toll-free (US) | +1.858.202.4566 tel
techsupport@illumina.com | www.illumina.com