

Grant Application Resources for Single Cell Multiome ATAC + Gene Expression

Summary

Transcriptome analysis at single cell resolution (single cell RNA-seq) has led to significant advances in our understanding of biology, elucidating cellular heterogeneity in complex systems and tissues, and enabling discovery of novel cell types, states, and biomarkers with relevance to clinical applications (1). However, single cell RNA-seq does not directly capture information about the drivers of gene regulation. This has left gaps in our understanding of how gene regulatory programs are established and ultimately how cell types and states are specified. To bridge this gap, innovative multiomic tools have emerged that simultaneously interrogate both gene expression and chromatin accessibility, enabling direct epigenetic and transcriptomic measurements from the same cell.

Efforts to link epigenetic and transcriptomic measurements from single cells have historically necessitated two separate assays. This requires two different input samples followed by inferred linkage between separate gene expression and chromatin accessibility (ATAC-seq) datasets. In addition to the computational challenges of accurately inferring and linking similar cells across samples, this approach may be affected by different cellular proportions between input samples and different sample preparation conditions that can influence data output and quality. Comparing computational inference of split single cell RNA-seq and ATAC-seq datasets with methods that directly link the two data types in single nuclei, Ma et al. found that accuracy was highly dependent on tissue (2).

To address these challenges, 10x Genomics has developed Chromium Single Cell Multiome ATAC + Gene Expression, a fully supported turnkey solution with assay and software components. This is the first commercially available solution for simultaneous capture of both transcriptomic and epigenomic modalities in the same single cells, across thousands of cells. This technique avoids the computational errors associated with linking separate datasets and maximizes possible readouts from precious samples. Moreover, with a unified view of a cell's open chromatin landscape and gene expression profile, it is possible to resolve how cell types and states are established, discover new gene regulatory interactions, and interpret epigenetic profiles with key expression markers.

Chromium Single Cell Multiome ATAC + Gene Expression

The Chromium Single Cell Multiome ATAC + Gene Expression workflow begins with a suspension of nuclei. Transposition is performed in bulk upon application of the enzyme transposase, which enters the nuclei and preferentially fragments the DNA in open regions of chromatin while adapter sequences are simultaneously added to the ends of the DNA fragments. Transposed nuclei are loaded onto a microfluidic chip, which is run in the Chromium Controller instrument.

In the instrument, nuclei are partitioned individually with a single Gel Bead forming droplets, or Gel Beads-in-emulsion (GEMs). Each Gel Bead contains oligonucleotides with a unique 16 base pair 10x Barcode sequence, a poly(dT) sequence to capture polyadenylated mRNA for a gene expression library, and a Spacer sequence that enables barcode attachment to transposed DNA fragments for an ATAC library. The unique 10x Barcode serves to associate mRNA and transposed DNA fragments back to the same nucleus. The gene expression library also carries an additional unique molecular identifier (UMI) to distinguish individual, captured mRNA molecules for quantification. The GEMs are then incubated to attach the unique barcodes to mRNA and transposed DNA fragments. This is followed by a reverse transcription reaction, converting mRNA into full-length cDNA.

Following this incubation, GEMs are broken and pooled fractions are recovered and purified. The product is taken through a pre-amplification PCR step to fill gaps and ensure maximum recovery of barcoded ATAC and cDNA.

fragments. Subsequently, the pre-amplified product is used as input for both ATAC library construction and cDNA amplification for gene expression library construction. Resulting libraries can be sequenced on next-generation short read sequencers at a recommended depth of 20,000 read pairs per cell for gene expression and 25,000 read pairs per cell for ATAC.

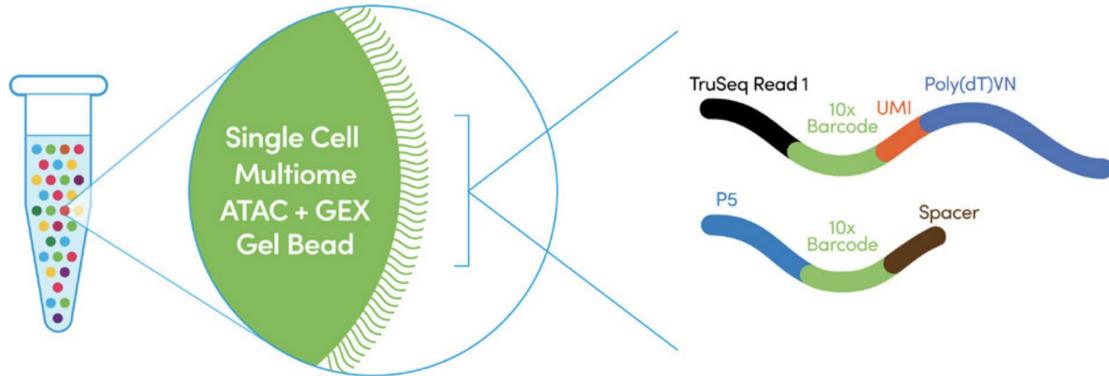
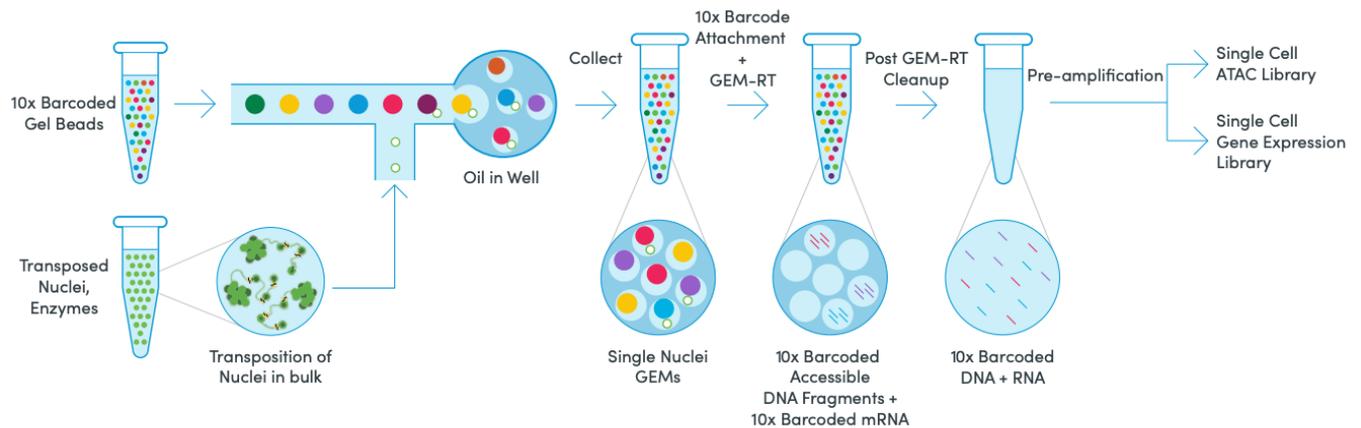


Diagram of Single Cell Multiome ATAC + Gene Expression (GEX) Gel Bead. Oligonucleotides containing a poly(dT) sequence to capture mRNA molecules and a Spacer sequence that enables barcode attachment are both carried on the Gel Bead. The unique 10x Barcode is used to associate the mRNA molecules and transposed DNA fragments back to the same nucleus after sequencing.



Efficient and robust workflow. Leverage a fully supported library preparation workflow and kitted reagents to streamline experimentation, providing a powerful approach to combine gene expression and ATAC-seq data from the same single cell.

Following sequencing, BCL or FASTQ files can be analyzed using the Cell Ranger ARC analysis pipeline and visualized using Loupe Browser. Both software tools are available for download on the 10x Genomics Support website. Together, they provide a view of the linkage of ATAC and gene expression data in the same single cell, across thousands of cells.

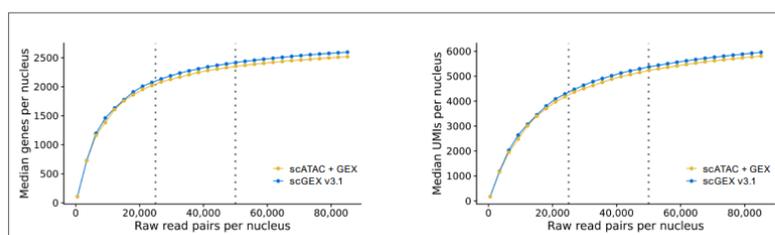
Cell Ranger ARC performs sample demultiplexing, barcode processing, identification of open chromatin regions, and simultaneous counting of transcripts and chromatin peak accessibility in single cells. Secondary analyses, such as dimensionality reduction, cell clustering, and differential gene expression and peak analysis, are also included. The

desktop visualization tool, Loupe Browser, enables interactive data exploration of cell clusters, differential accessibility or gene expression, and feature linkages between open chromatin regions and gene expression patterns to aid in data interpretation.

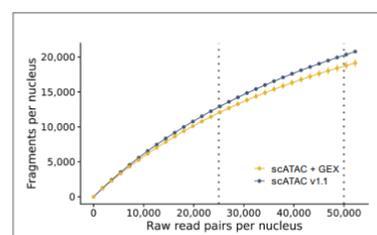
Validation studies and data benchmarking

In the data highlighted below, nuclei from mouse embryonic brain samples were processed with Chromium Single Cell Multiome ATAC + Gene Expression and the independent gene expression and ATAC assays, Chromium Single Cell Gene Expression (v3.1) and Chromium Single Cell ATAC (v1.1). Data showed that both gene expression sensitivity, as measured by median genes per nucleus and median UMIs per nucleus, and ATAC sensitivity, as measured by median fragments per nucleus, are comparable between the individual assays and Chromium Single Cell Multiome ATAC + Gene Expression.

A. Gene Expression



B. ATAC



Generate high-quality single cell gene expression and ATAC libraries. Mouse embryonic E18 brain samples were processed using Chromium Single Cell Gene Expression, Chromium Single Cell ATAC, and Chromium Single Cell Multiome ATAC + Gene Expression. Analysis of gene expression data included sequencing reads mapping to introns. Sensitivity of gene expression or ATAC signals was determined across a range of read depths using in silico downsampling. **A.** Gene expression sensitivity, as measured by median genes per nucleus or median UMIs per nucleus, is comparable between Single Cell Gene Expression v3.1 and Single Cell Multiome ATAC + Gene Expression. **B.** Similarly, ATAC sensitivity, as measured by high-quality unique fragments per nucleus, is comparable between Single Cell ATAC v1.1 and Single Cell Multiome ATAC + Gene Expression.

Applications

Chromium Single Cell Multiome ATAC + Gene Expression enables deep cellular characterization through investigation of the gene regulatory networks that control cell identity, state, and function. Among many applications that span diverse research fields, the technology can be used to:

- [Resolve cancer cell-specific gene regulatory programs and associated gene expression signatures \(3\)](#)
- [Define gene regulatory programs driving neural differentiation in development and disease \(4\)](#)
- [Deeply characterize immune cell populations and uncover T-cell states hidden by single-parameter readouts \(5\)](#)

Advantages

Chromium Single Cell Multiome ATAC + Gene Expression offers many technical advantages, making it an optimal product for single cell transcriptomic and epigenetic characterization. These include:

- Accurate data linkage—Integrated gene expression and epigenomic profiling through direct measurement in the same cell, eliminating the need for inferring relationships in silico.

- Simple and robust workflow—Efficiently partition 500–10,000 nuclei per channel, for up to 80,000 nuclei per run and the ability to run up to 8 samples in parallel.
- Validated results—Recover paired gene expression and chromatin accessibility profiles for up to 65% of loaded nuclei for a high-sensitivity solution with a low microfluidic multiplet rate (<1% per 1000 nuclei).
- Optimized conditions for diverse samples—Demonstrated with cell lines, primary cells, cryopreserved cell suspensions, and fresh and flash-frozen tissue.
- Comprehensive data analysis solution—Chromium Single Cell Multiome ATAC + Gene Expression includes an easy-to-use data analysis pipeline as well as state-of-the-art software for data visualization. This enables streamlined interpretation of epigenetic profiles with key expression markers, including identification of linkages between putative regulatory elements and their target genes.
- Broad support resources—10x Genomics provides comprehensive support resources, ranging from technical specialists trained in the Chromium Single Cell Multiome ATAC + Gene Expression workflow to freely available [videos](#) and [documents](#) that guide users through the workflow.
- Certified product quality—10x Genomics product development and manufacturing processes are ISO 9001:2015 certified.

Additional Resources

For more information about Chromium Single Cell Multiome ATAC + Gene Expression, explore these helpful resources with product specifications, including the list of kits required to run the assay, data benchmarking, sample prep guidance, and answers to common questions.

- Chromium Single Cell Multiome ATAC + Gene Expression [Product Sheet](#)
- Answering Your Questions About Chromium Single Cell Multiome ATAC + Gene Expression [Blog Post](#)
- Chromium Next GEM Single Cell Multiome ATAC + Gene Expression Data Comparison [Technical Note](#)
- Nuclei Isolation Demonstrated Protocols for:
 - [Cell lines and PMBCs](#)
 - [Mouse embryonic brain](#)
 - [Complex tissues](#)

References

1. Stark R, et al. RNA sequencing: the teenage years. *Nat Rev Genet* 20: 631–656, 2019.
2. Ma S, et al. Chromatin potential identified by shared single-cell profiling of RNA and chromatin. *Cell* 183: 1103–1116.e20, 2020.
3. Hung K, et al. EcDNA hubs drive cooperative intermolecular oncogene expression. *bioRxiv*, 2020.
4. Trevino A, et al. Chromatin and gene-regulatory dynamics of the developing human cerebral cortex at single-cell resolution. *bioRxiv*, 2021.
5. Hao Y, et al. Integrated analysis of multimodal single-cell data. *bioRxiv*, 2020.