

Grant Application Resources for GEM-X Epi Multiome

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, epigenetic tools like single cell ATAC-seq (Assay for Transposase-Accessible Chromatin using Sequencing) enabled researchers to capture chromatin accessibility and profile active regulatory elements on a cell-by-cell basis.

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 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 GEM-X Epi Multiome, a fully supported turnkey solution with assay and software components. This is the next evolution of the first commercially available solution for simultaneous capture of both transcriptomic and epigenomic modalities in the same single cells, across hundreds of thousands of cells. This technique maximizes possible readouts from precious samples and enables direct links between scRNA-seq and scATAC-seq data, avoiding the computational errors associated with linking separate datasets. 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.

GEM-X Epi Multiome

The GEM-X Epi Multiome 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 X series instrument.

In the instrument, nuclei are partitioned individually in nanoliter-sized reaction vessels (or droplets) known as 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 nucleus for gene expression and 25,000 read pairs per nucleus for ATAC.

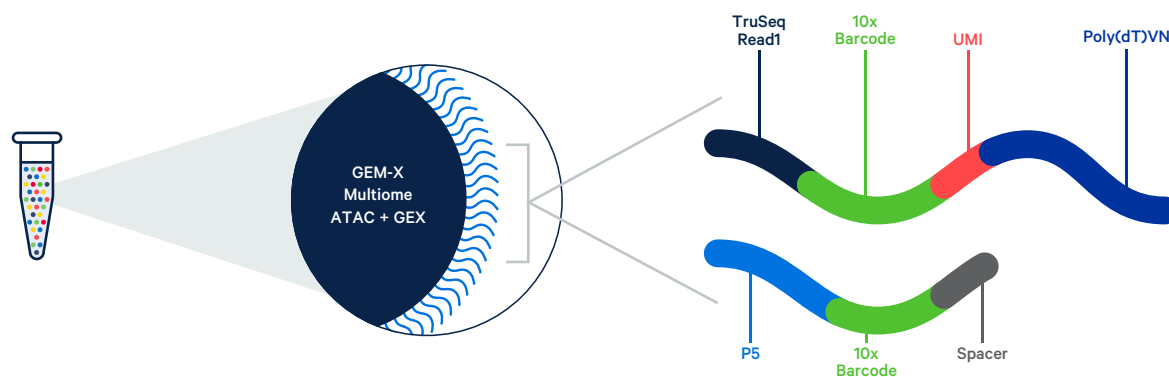
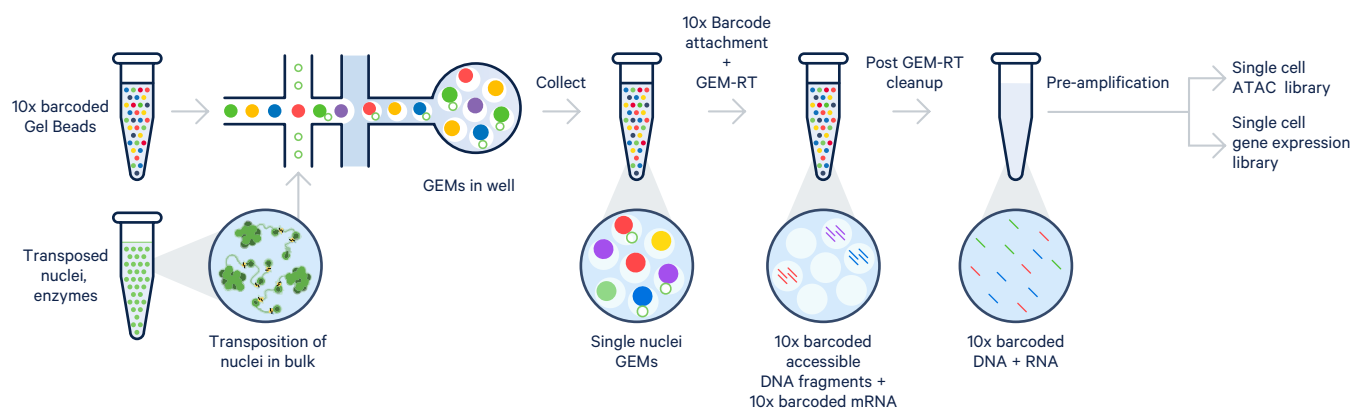


Diagram of a GEM-X Multiome ATAC + GEX Gel Bead. Oligonucleotides containing a poly(dT) sequence to capture mRNA molecules and a Spacer sequence that enables barcode attachment are both carried on the Gel Bead. The unique 10x Barcode is used to associate the mRNA molecules and transposed DNA fragments 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 simultaneously measure gene expression and ATAC-seq data from the same single cell.

Following sequencing, 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. Additionally, the Cell Ranger ARC pipeline is easily accessible via the 10x Genomics Cloud Analysis web interface. Together, these analysis tools provide a view of the linkage of ATAC and gene expression data in the same single cell, across hundreds of thousands of cells.

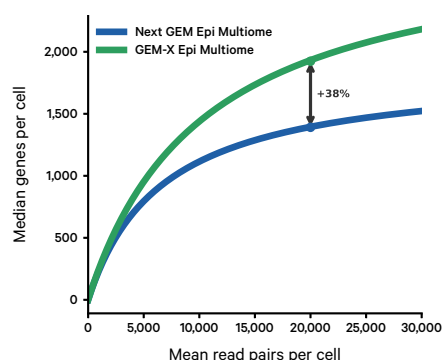
Cell Ranger ARC software performs barcode processing, identification of open chromatin regions, and simultaneous counting of transcripts and chromatin peak accessibility in single cells. Secondary analyses, such as dimensionality reduction, cell clustering, and differential gene expression and peak analysis,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

Data benchmarking

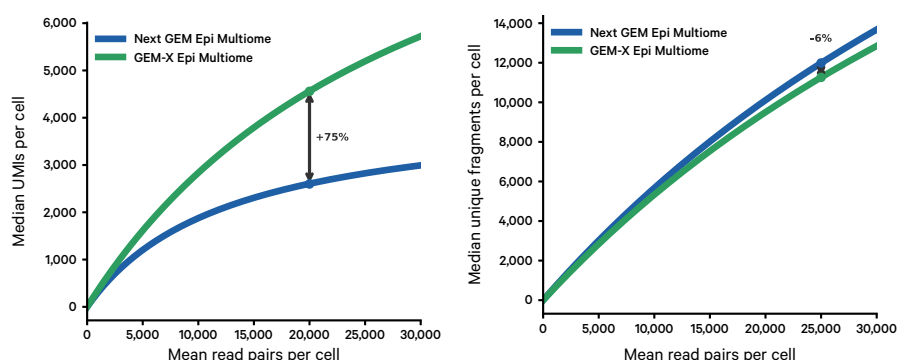
Since the initial commercial release of the Chromium Next GEM Epi Multiome ATAC + Gene Expression assay in 2020, it has been widely adopted and data has been published in over 300 peer-reviewed publications across research areas including: oncology, neuroscience, developmental biology, and immunology.

In the data highlighted below, nuclei from human brain samples were processed with the Next GEM Epi Multiome ATAC + Gene Expression and the next generation GEM-X Epi Multiome assays. Data show that gene expression sensitivity, as measured by median genes per nucleus and median UMIs per nucleus, are significantly increased for samples processed with the GEM-X Epi Multiome assay. While the ATAC sensitivity, as measured by median unique fragments per nucleus, shows similar performance. Similar gene expression and ATAC performance trends were seen across tissue types tested, including: human lung, human prostate cancer, mouse brain, mouse lung.

A. Gene Expression



B. ATAC



GEM-X Epi Multiome out performs Next GEM Multiome with higher gene expression sensitivity while maintaining similar ATAC sensitivity.

Human brain samples were processed using the Next GEM Multiome ATAC + Gene Expression and GEM-X Epi Multiome assays. 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 greater for GEM-X Epi Multiome compared to the Next GEM assay. **(B)** ATAC sensitivity, as measured by high-quality unique fragments per nucleus, was similar for GEM-X Epi Multiome compared to the Next GEM assay.

Applications

GEM-X Epi Multiome 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

GEM-X Epi Multiome 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 from the same cell, eliminating the need for inferring relationships in silico.
- **Robust workflow**—Efficiently partition 500–20,000 nuclei per channel, for up to 160,000 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 80% of loaded nuclei for a high-sensitivity solution with a low microfluidic multiplet rate (<0.5% per 1,000 nuclei).
- **Optimized conditions for diverse samples**—Demonstrated with cell lines, primary cells, cryopreserved cell suspensions, and fresh and flash-frozen tissue. The Nuclei Isolation Kit with RNase Inhibitor v2 further streamlines nuclei isolation from tissues.
- **Comprehensive data analysis solution**—GEM-X Epi Multiome 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 GEM-X Epi Multiome workflow to freely available [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 GEM-X Epi Multiome, 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.

- GEM-X Epi Multiome [Product Sheet](#)
- Nuclei Isolation Kit with RNase v2 [User Guide](#)
- Nuclei Isolation Demonstrated Protocols for:
 - [Cell lines and PMBCs](#)
 - [Complex tissues](#)
- Cell Ranger ARC data analysis [software](#)

References

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4. Sojka C, et al. Mapping the developmental trajectory of human astrocytes reveals divergence in glioblastoma. *Nat Cell Biol* 27: 347–359 (2025). doi: 10.1038/s41556-024-01583-9
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