Grant application resources for GEM-X Universal 3' Gene Expression

Summary statement

Each of the assays in the Chromium Single Cell platform are developed for specific research needs. Here, we discuss the unique benefits of our GEM-X Universal 3' Gene Expression (v4) assay, which supports a reverse transcriptase–based workflow to measure whole transcriptome gene expression and cell surface protein levels at single cell resolution. For years, our Universal 3' Gene Expression assay (formerly known as Chromium Single Cell Gene Expression) has empowered many researchers to make key discoveries in oncology (1–3), neuroscience (4–6), immunology (7–9), developmental biology (10–11), and drug discovery and development (12–13). GEM-X Universal 3' Gene Expression improves upon our tried-and-tested workflow, offering a cost-effective single cell solution with exceptional sensitivity and robustness. As more and more researchers continue to adopt scRNA-seq, 10x Genomics will continue to build new features and applications for our single cell platform to address the ever-expanding needs of researchers and propel research forward.

Chromium platform overview

The Chromium platform from 10x Genomics offers a suite of products enabling analysis of transcriptomes, chromatin accessibility, paired B- and T-cell receptor sequences, and proteins on a cell-by-cell basis. Researchers have used these trusted, reliable assays to significantly advance scientific knowledge, publishing over 9,100 publications. The publications cover analysis of a variety of tissue types and species in a breadth of fields. 10x Genomics is committed to making our robust Chromium assays accessible to researchers with different skill sets. These assays are supported by instruments that automate critical stages in every protocol, ensuring researchers with varied experience can obtain accurate, reproducible results. Additionally, researchers with or without bioinformatics experience can use our suite of analysis tools and pipelines—including Cell Ranger software and Loupe Browser—to process, visualize, and explore their data. Researchers also have access to 10x Genomics technical experts who can provide support through scientific and technical consultations, workflow optimization, and methodology troubleshooting. Researchers in need of further support can utilize a global network of Contract Research Organizations (CROs) and Service Providers (SPs) who can assist with every stage of our work flow from sample preparation to library generation—and the specific needs of drug discovery researchers, including global sample acquisition and management.

GEM-X Universal 3' Gene Expression (v4) overview

Single cell RNA sequencing (scRNA-seq) is a well-established, reliable method that has enabled researchers to delve into the biological complexities driven by cellular heterogeneity in a way other methods—including bulk RNA-seq, flow cytometry, and mass spectrometry—cannot.

For example, traditional bulk RNA-seq provides the average gene expression in multicellular tissue, leaving researchers to infer the proportion of known cell types, cell-to-cell variability in gene expression, and more. Rare cell states and populations are often masked in these datasets. Additionally, traditional methods to analyze intracellular and cell surface protein levels often fail to capture these unique cell populations, especially transient cell

states—such as differentiating cells—where genes are undergoing rapid and dynamic regulation before entering a stable cell state with well-defined protein markers. scRNA-seq has allowed researchers to identify these short-lived cell states, leading to key discoveries in early human development (10) and more.

To address these limitations, 10x Genomics developed Universal 3' Gene Expression—now powered by GEM-X technology and supported by the Chromium X series instrument—which enables researchers to profile tens of thousands of genes at single cell resolution for unbiased characterization of cell types and states, alongside detection of hundreds of cell surface proteins for ultra-high parameter multiomic cytometry.

GEM-X Universal 3' Gene Expression (v4) standard workflow

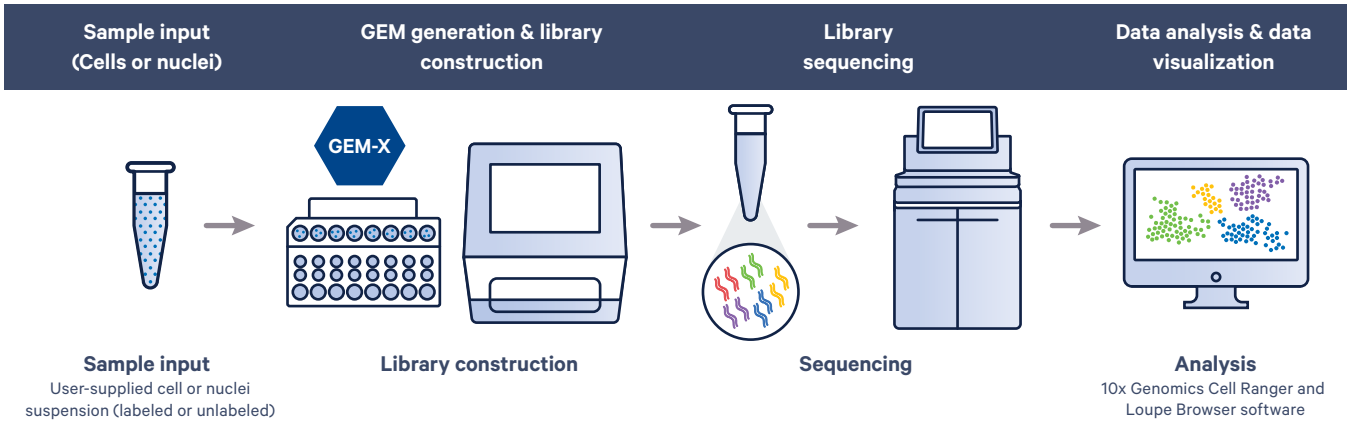

Fig 1. Schematic overview of the GEM-X technology workflow. A typical workflow starts with user-supplied cells or nuclei that are combined with our GEM-X reagents and consumables, enabling partitioning, cell lysis, and barcoding of cellular information on a Chromium X series instrument. These barcoded transcripts are then amplified and converted into libraries compatible with Illumina or other short-read sequencing platforms. After sequencing, the results are analyzed using the Cell Ranger pipeline and visualized using Loupe Browser.

With GEM-X Universal 3' Gene Expression (v4), transcriptomes are analyzed on a cell-by-cell basis through the use of microfluidic partitioning to capture single cells and prepare barcoded, next-generation sequencing (NGS) libraries (Figure 1). The workflow starts with a suspension of single cells or nuclei. The suspension is mixed with lysis buffer; Gel Beads are coated with oligonucleotides, which include a 10x Barcode that marks each RNA molecule's cell of origin; a unique molecular identifier (UMI) that gives each transcript a unique fingerprint; a poly(dT) sequence for capturing mRNA at the 3' end; and adapter sequences for downstream next-generation sequencing (Figure 2).

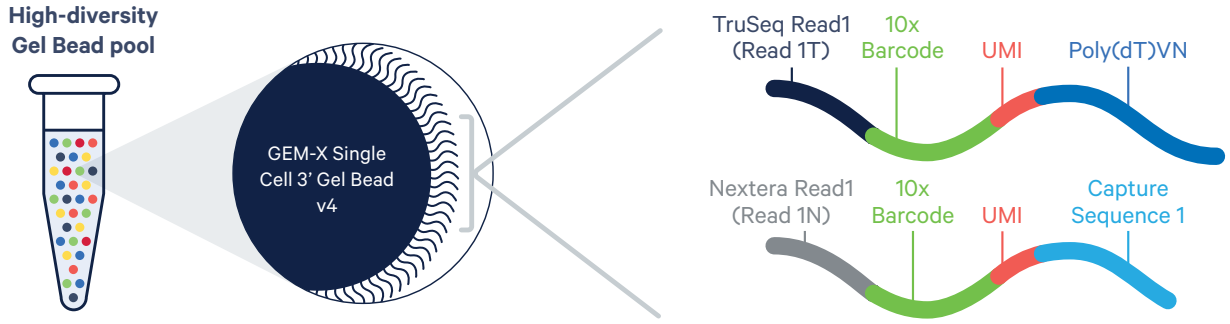

Figure 2. Schematic diagram of a GEM-X Single Cell 3' Gel Bead. Every Gel Bead is coated with oligos containing an Illumina TruSeq Read 1 (Read 1 sequencing primer, Read 1T), 16-nucleotide (nt) 10x Barcode, 12-nt unique molecular identifier (UMI), and 30-nt poly(dT)VN.

Once a loaded microfluidic chip is placed in the Chromium X series instrument, cells move through the channels at a limiting dilution to generate nanometer-sized reaction vessels known as Gel Beads in Emulsion (GEMs; Figure 3). The cell is lysed and the Gel Bead dissolves. The 3' end of the released RNA binds to the poly-dT sequences that coated the Gel Bead. The primed RNA is then reverse transcribed, forming complementary DNA (cDNA).

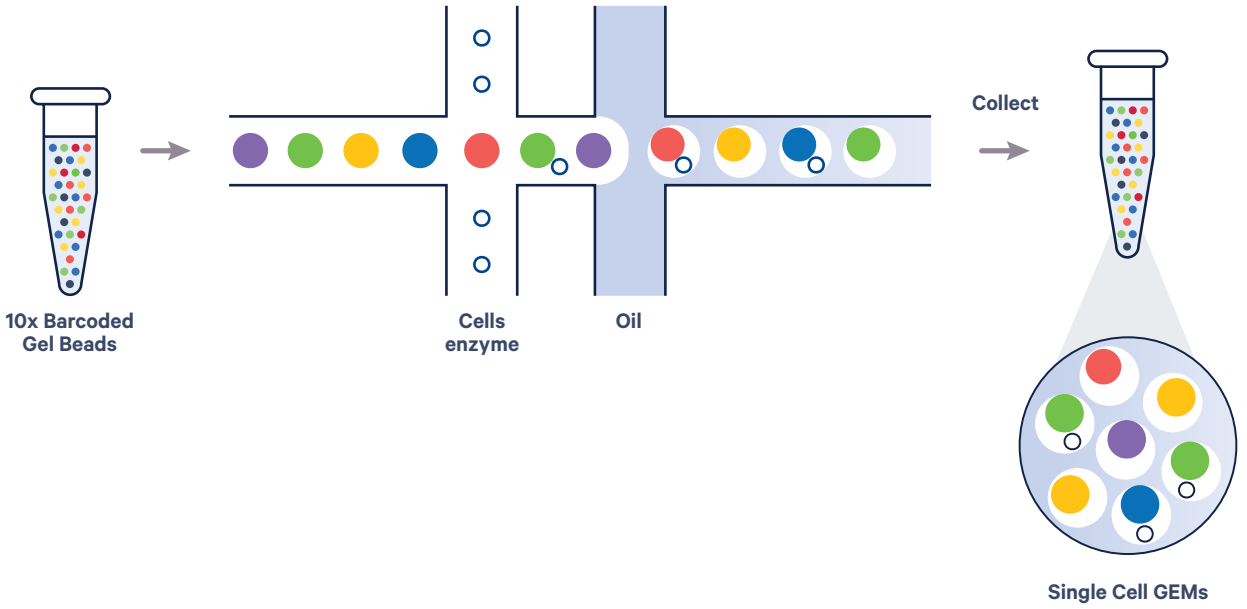

Figure 3. A schematic overview of GEM generation and barcoding with the GEM-X chip workflow. GEMs are generated by combining barcoded Gel Beads, a master mix containing cells, and partitioning oil in a GEM-X 3' Chip. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90–99%) of generated GEMs contain no cell, while the remainder largely contain a single cell.

All cDNAs from a single cell, originating from a single GEM, will have the same 10x Barcode, allowing the user to map each transcript back to its cell of origin. The individual oligos on a single Gel Bead, however, have different UMIs, which distinctly mark each cDNA molecule with a unique, random 12-base sequence. This allows the user to eliminate any PCR duplicates during downstream processing. The barcoded cDNA is then used to prepare a sequencing library, which includes the addition of sequencing adapters for PCR amplification. Finally, the library is sequenced with NGS.

Advanced GEM-X technology

GEM-X technology offers optimized reagents, improved microfluidics, and upgraded analysis software to drive improvements to every step of the tried-and-tested Universal 3' Gene Expression workflow. Specifically, GEM generation and cell partitioning are more efficient than the previous iteration of this assay, Next GEM Universal 3' Gene Expression (v3.1).

The previous version is supported by Next GEM technology in which GEMs are generated independent of partitioning oil via step emulsification. GEM-X microfluidics utilize the flow of partitioning oil to facilitate GEM formation. With this new technology, twice as many GEMs are generated at smaller volumes, reducing multiplet rates two-fold and increasing throughput capabilities. Additionally, cell partitioning and barcoding are faster and more robust, resulting in an abbreviated six-minute run time.

The Chromium X series instruments maximize these improvements by automating cell partitioning and barcoding, minimizing the risk of technical error between researchers of varying skill levels. Even researchers using samples drawn from large clinical or translational studies across multiple institutions have reported robust results (14). (See Figure 10 for data demonstrating high inter- and intra-chip reproducibility in the Validation Studies section below.) Furthermore, advancements to the underlying chemistry of these automated steps in the GEM-X Universal 3' Gene Expression (v4) assay compared to the Next GEM Universal 3' Gene Expression (v3.1) assay translate to key performance improvements:

- Increased sensitivity enabled by a two-fold increase in detected genes and improved detection of rare transcripts, fragile cells, and cells with low RNA content (Figure 4)
- Higher throughput with a two-fold increase in cells captured per channel (up to 20,000 cells per channel)
- More cost-effective experiments, with a more than 50% reduction in cost per cell
- Improved sample recovery—up to 80% cell recovery efficiency (Figure 5), minimized clogging, and maximized cell size flexibility
- Enhanced data quality and robustness due, in part, to a halved multiplet rate (0.4% per 1,000 cells) and more efficient GEM generation with no increase in cell stress
- Faster run times—tens of thousands of cells are partitioned and barcoded in just 6 minutes

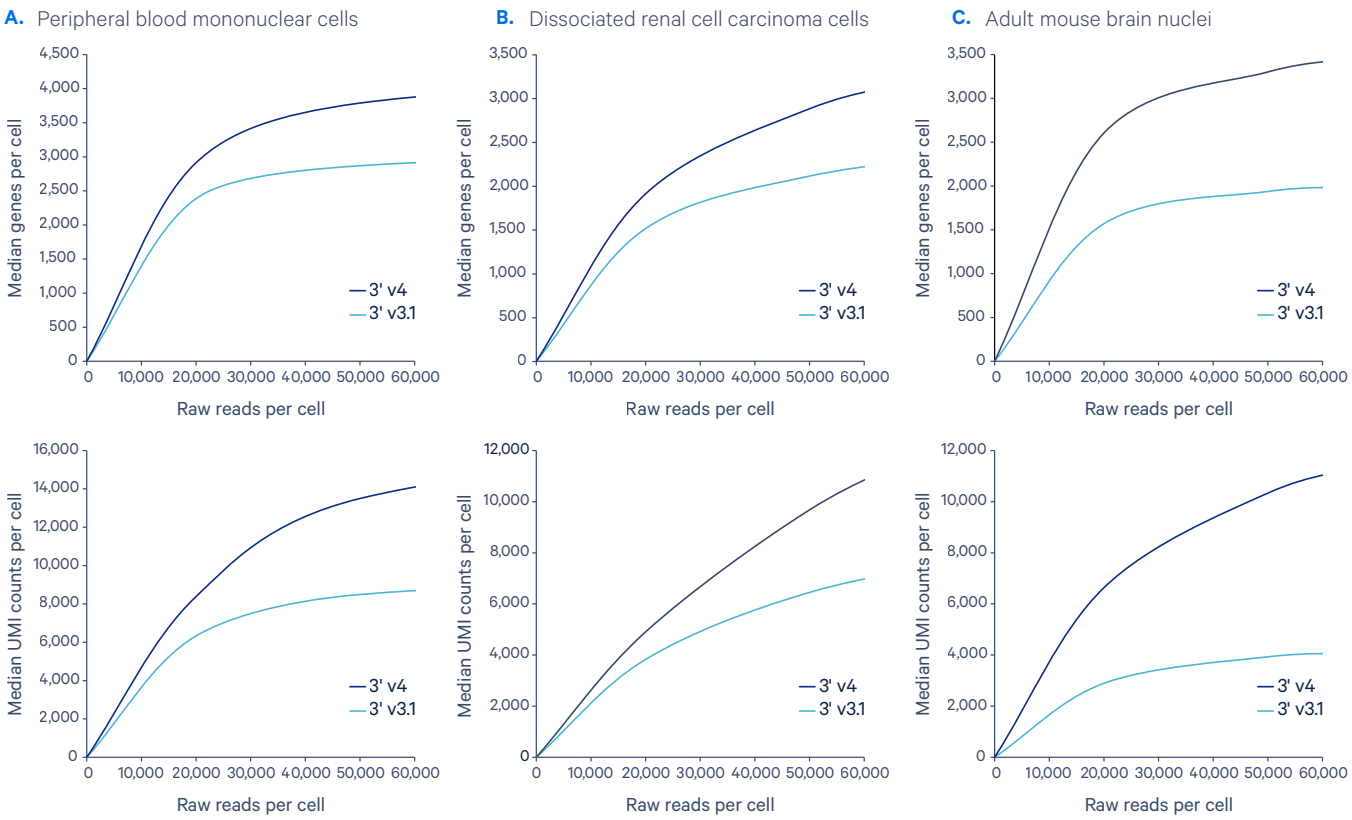

Figure 4. Substantial increase in library complexity with GEM-X Universal 3 Gene Expression v4 across three complex sample types. Sample types tested are as follows: PBMCs (**A**), dissociated cells obtained from renal cell carcinoma tissue (**B**), and nuclei obtained from an adult mouse brain (**C**). Each sample type was run in duplicate.

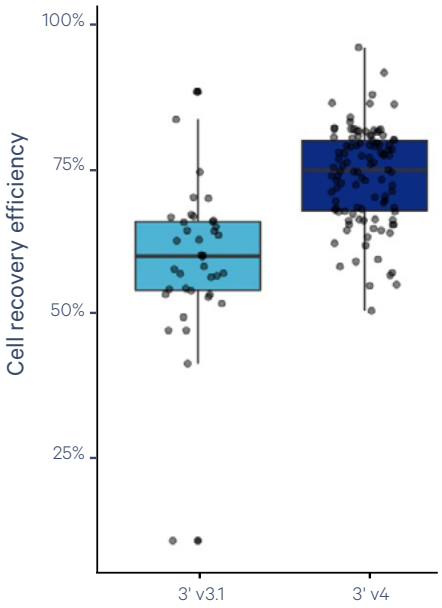

Figure 5. Improved cell recovery efficiency with GEM-X. Cell recovery efficiency was assessed with 318 independent single cell runs. Targeted cell loads ranged between 1,000–10,000 cells. Median cell recovery efficiency for GEM-X (3' v4) was 15% higher compared to Next GEM (3' v3.1).

GEM-X Universal 3' Gene Expression Multiplex with on-chip multiplexing workflow

GEM-X Universal 3' Gene Expression Multiplex is a separate offering that uses the same high-performance chemistry as our standard GEM-X Universal 3' Gene Expression and enables on-chip multiplexing (OCM). With OCM microfluidics, researchers have access to a simple and reliable workflow that allows batching of 4 independent samples per set (up to 8 samples per chip) and collection of all partitioned cells in the same recovery well. OCM does not involve tagging of samples upstream of chip loading, thus providing compatibility for diverse species and an easy, streamlined protocol. This method reduces reagent volumes for more cost-effective studies (even at small scales) and eases technical restrictions for multiplexing of limited samples like stem cells or flow-sorted cells by lowering cell input requirements.

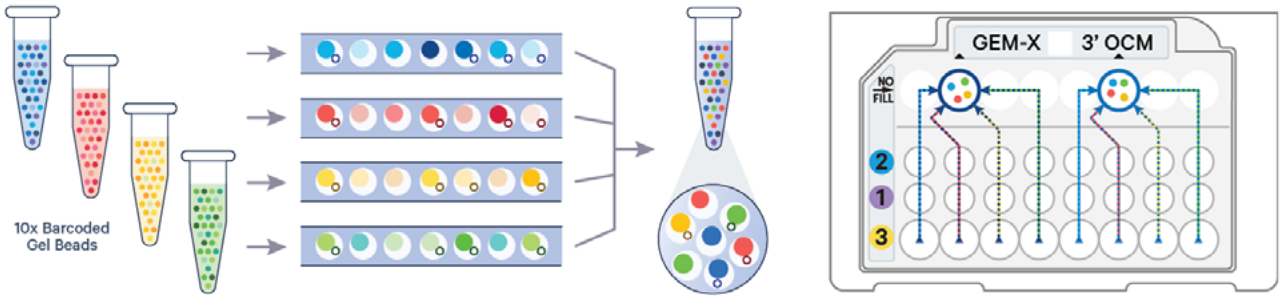

Figure 6. On-chip multiplexing with GEM-X Universal 3' Gene Expression Multiplex. Cells from up to 4 independent samples are co-partitioned, and all GEMs are then batched and collected in the same recovery well. Up to 8 samples can be loaded per chip with no sample tagging required upstream of chip loading. Samples are demultiplexed computationally after sequencing.

GEM-X Universal 3' Gene Expression with Feature Barcode technology workflow

Transcriptomic analysis provides a view on both current cell types and potential states, while cell surface protein readouts provide insight into the cell's current cell type and functional state. Combining transcriptome and proteome datasets from individual cells not only allows researchers to refine definitions of cell types and functional states for individual cells, but it also improves the resolution of transcriptionally similar cell subtypes, increasing the chances of discovering novel cell subtypes and rare cell subpopulations.

The GEM-X Universal 3' Gene Expression (v4) product supports single cell multiomic phenotyping through the simultaneous profiling of hundreds of cell surface proteins with Feature Barcode technology alongside 3' gene expression analysis. The multiomic protocol follows the same general workflow as the standalone 3' v4 assay (Figure 1), with a few key modifications to sample preparation (Figure 7), the micro-reactions that occur within the GEMs (Figure 8), and the dual index libraries (Figure 9). Cells within the single cell suspension are labeled with oligo-conjugated antibodies. The antibody-conjugated DNA barcodes are amplified within the GEMs for protein detection.

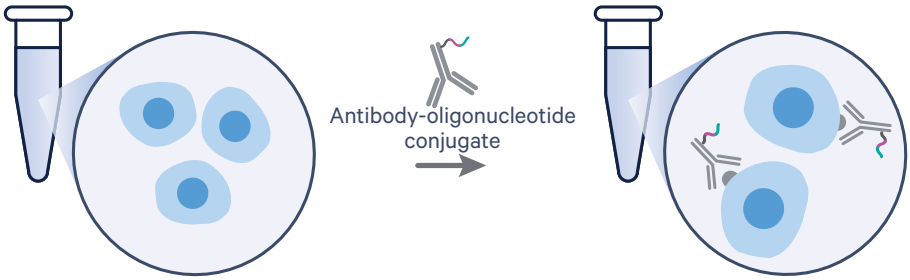

Figure 7. Sample preparation with Feature Barcode technology. Antibody-oligonucleotide conjugates can be added during sample preparation to label cell surface proteins of interest, enabling simultaneous analysis of gene and protein expression.

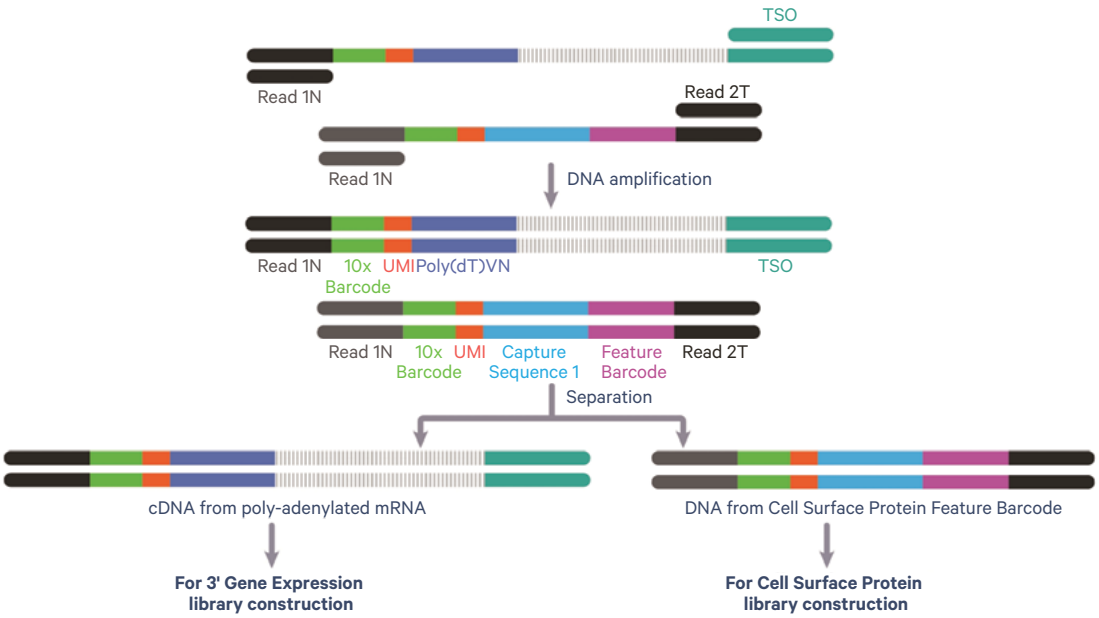

Figure 8. Pooled cDNA amplification. After incubation, GEMs are dissolved and pooled fractions are recovered. Silane magnetic beads are used to purify the cell-barcoded products from the post GEM-RT reaction mixture, which includes leftover biochemical reagents and primers. The cell-barcoded cDNA molecules are amplified via PCR to generate sufficient mass for library construction. Size selection is used to separate the amplified cDNA molecules for 3' Gene Expression and Cell Surface Protein library construction.

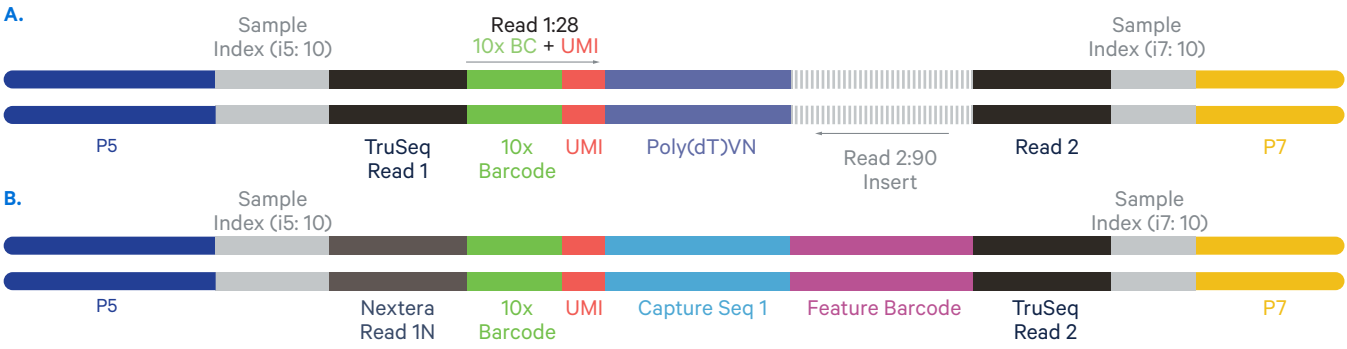

Figure 9. Chromium GEM-X Single Cell 3' Dual Index libraries. A. Chromium GEM-X Single Cell 3' Gene Expression Dual Index library comprises standard Illumina paired-end constructs that begin and end with P5 and P7. The 16-nt 10x Barcode and 12-nt UMI are encoded in Read 1, while Read 2 is used to sequence the cDNA fragment. **B.** A Chromium GEM-X Single Cell 3' Cell Surface Protein Dual Index library comprises standard Illumina paired-end constructs that begin and end with P5 and P7. The 16-nt 10x Barcode and 12-nucleotide UMI are encoded in Read 1, while Read 2 is used to sequence the antibody-conjugated DNA barcode.

Data analysis

10x Genomics software tools provide researchers with varying levels of bioinformatics experience access to an intuitive analysis pipeline. Cell Ranger and Loupe Browser software—available on the 10x Genomics Support website—enable users to analyze the sequencing data from GEM-X Universal 3' Gene Expression analyses.

Data analysis starts with Cell Ranger software, which allows researchers to:

- Process raw sequencing data to align reads and perform quality control analyses
- Identify cell barcodes to quantify gene and protein expression levels in individual cells
- Demultiplex samples from OCM workflows for GEM-X Universal 3' Gene Expression Multiplex
- Generate cell feature matrices to analyze data produced using multiomic assays, such as GEM-X Universal 3' Gene Expression with Feature Barcode technology
- Perform clustering and secondary analyses, such as dimensionality reduction, cell clustering, and differential gene expression
- Automatically identify and annotate cell types based on standardized datasets with our Automated Cell Annotation* tool

Additionally, our trusted Cell Ranger pipelines are easily accessed via the 10x Genomics Cloud Analysis platform, a simple web interface that processes your data in as little as 90 minutes.

The desktop visualization tool, Loupe Browser, enables interactive data exploration of cell clusters and differential gene expression to aid data interpretation. With Loupe Browser, you can:

- Find significant genes—Determine genes that uniquely characterize clusters at the press of a button
- Refine cell types—Use gene lists and the gene expression view to refine cell types from Cell Ranger Automated Cell Annotation
- Assess data quality—Compare clustering resolution and performance across samples

*Annotation is performed using a model that was co-developed by 10x Genomics and the Cellarium AI Lab at the Data Sciences Platform of the Broad Institute.

Researchers can integrate data produced using our 3' v3.1 (Next GEM) and 3' v4 (GEM-X) assays with chemistry batch correction, preventing interruptions to ongoing projects that include personal or publicly available datasets produced with 3' v3.1.

If further analysis is needed, researchers can seamlessly move between Loupe Browser and community-developed tools written in R using the conversion package, LoupeR. LoupeR also eases collaboration with external collaborators or service providers that offer researchers in-depth bioinformatics expertise, including 10x Genomics Service Providers (SPs).

Validation studies

The GEM-X 3' microfluidic chip has higher inter- and intra-chip reproducibility. To evaluate reproducibility across multiple channels and chips, a single user loaded a suspension of cryopreserved human PBMCs across all eight channels of a GEM-X chip and proceeded through the remaining workflow steps. This experiment was then repeated each week for a total of three weeks. Clustering and cell-type populations were highly concordant across chip channels and experimental weeks, underscoring the high inter- and intra-chip reproducibility of our microfluidic technology and assays, further evidenced by high UMI correlation scores (R2 >0.96) achieved across the aggregated data for each of the three weeks of this experiment (Figure 10).

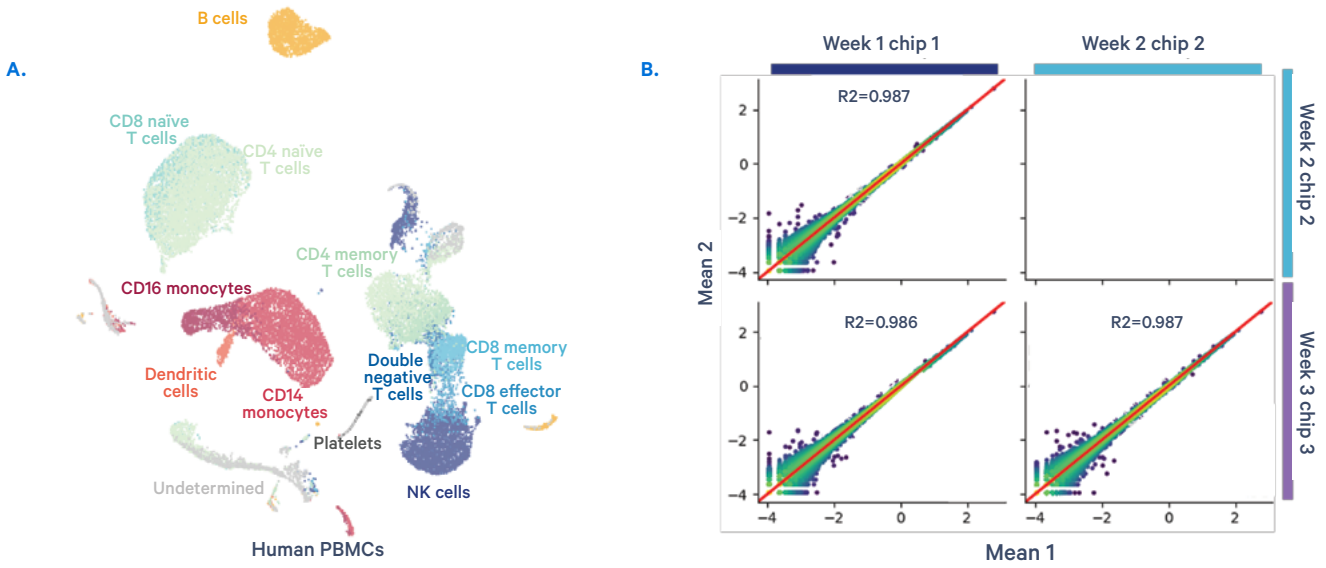

Figure 10. GEM-X microfluidic chips have high inter- and intra-chip reproducibility. A. An aggregated overlay of the data collected through analysis of three chips, each run one week apart (n = 24 channels; 26,191 cells) by one individual with all major immune cell populations identified. **B.** Mean UMI comparison scores across experimental weeks.

The changes to the chemistry driving the GEM-X Universal 3' Gene Expression (v4) assay did not impact the cell populations observed following analysis of well-characterized tissue samples (Figure 11). In fact, the performance enhancements gained with GEM-X technology have demonstrated enhanced capabilities to detect challenging cells (Figure 12). For example, neutrophils are notoriously challenging to capture for scRNA-seq analysis due to their fragile nature, low RNA content, and, potentially, the presence of interfering proteases and nucleases in neutrophil granules—a factor that makes all granulocytes difficult to capture (15). Researchers often modify standard scRNA-seq workflows to improve capture of these cells, but GEM-X Universal 3' Gene Expression efficiently captures these cells with its standard workflow (Figure 12).

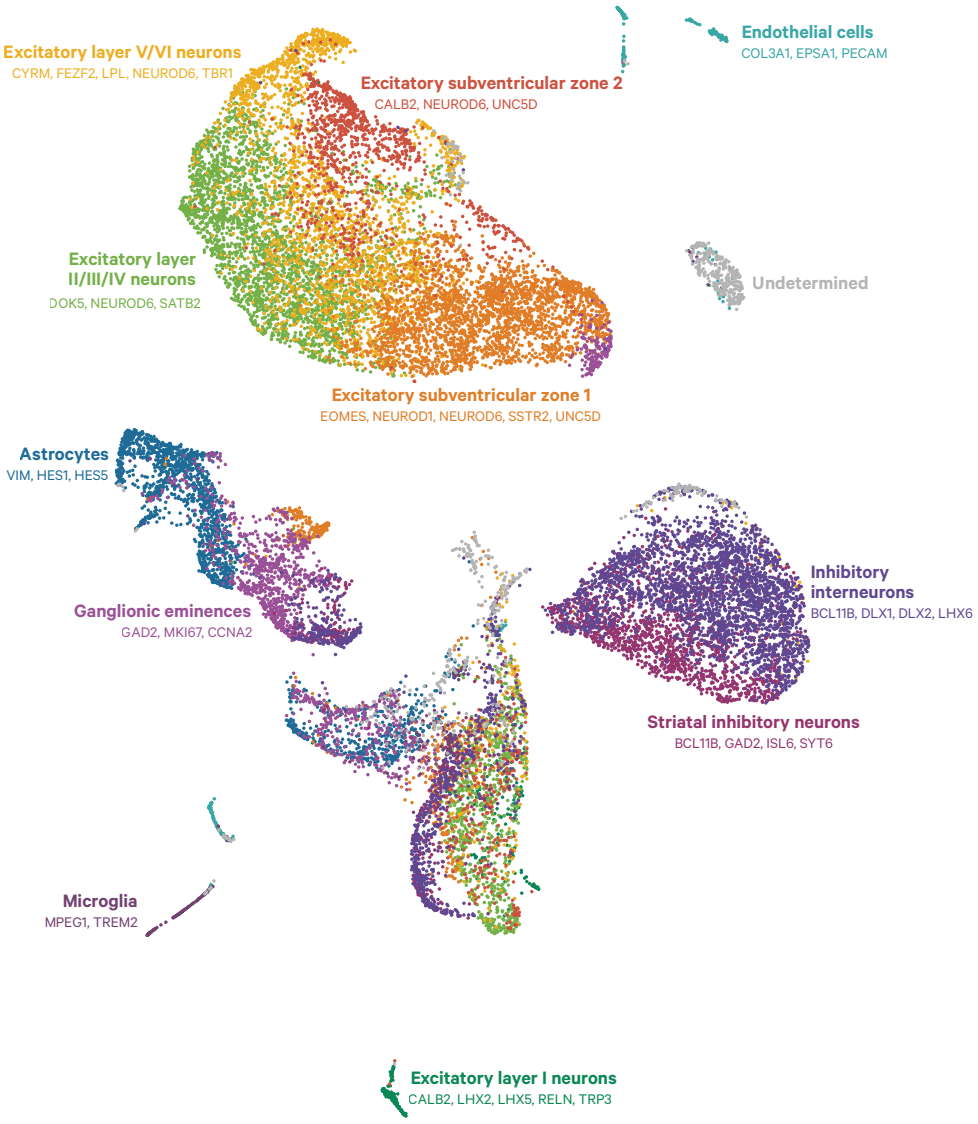

Figure 11. Reveal heterogeneous cell composition of complex tissue with single cell gene expression, powered by GEM-X. Aggregated UMAP projection of 21,331 cells from eight technical replicates profiled with the GEM-X Universal 3' Expression v4 solution. Cells were isolated from dissociated embryonic mouse brain tissue (E18 mouse combined cortex, hippocampus, and ventricular zone). Each cell is represented by a single dot. Broad categories of neuronal populations were identified through canonical gene expression markers.

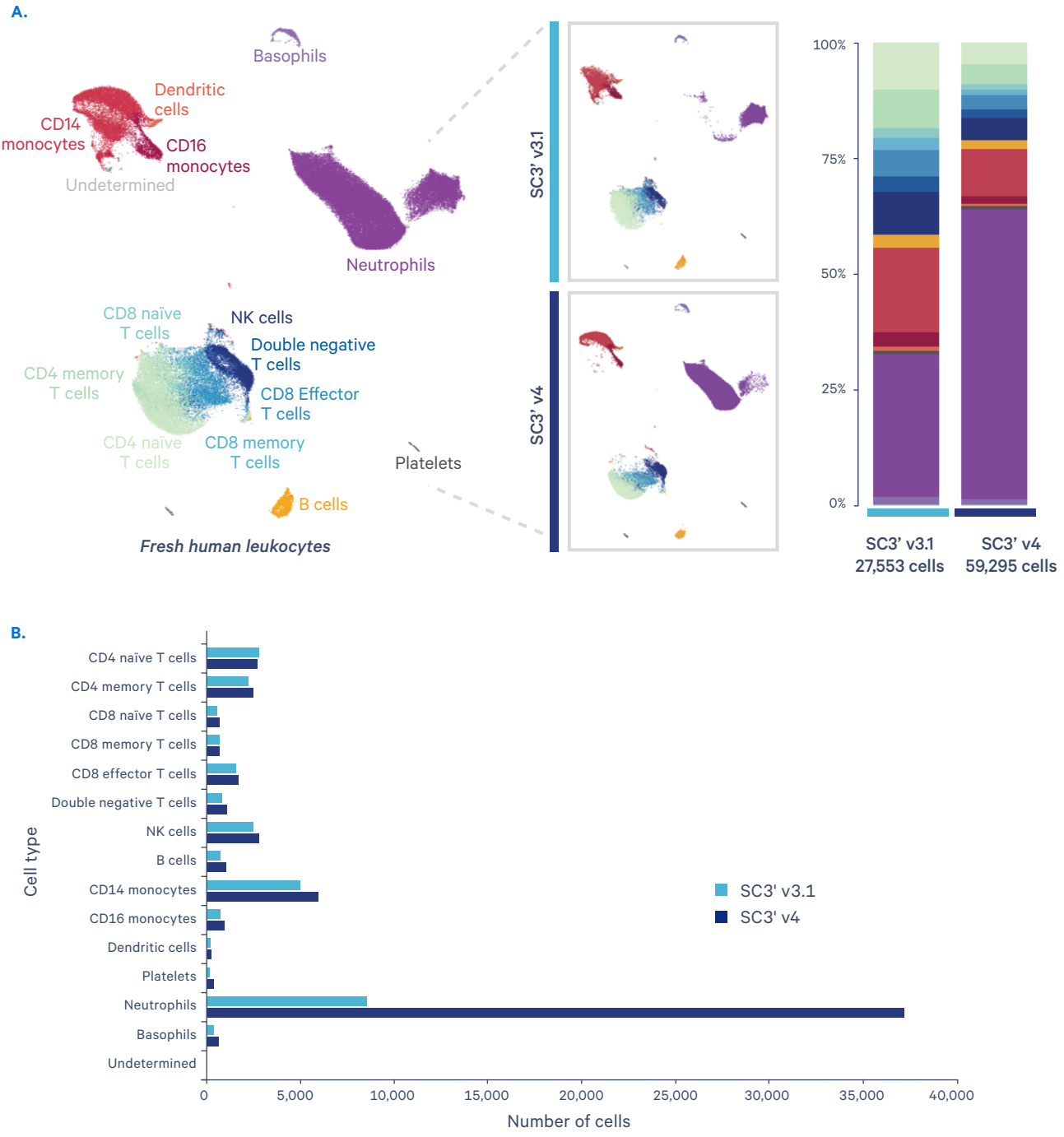

Figure 12. GEM-X Universal 3' Gene Expression v4 (SC3' v4) improves detection of challenging cell types, including neutrophils. A. Freshly isolated leukocytes were loaded separately onto a Next GEM Chip G (n = 8 channels for a total of 27,553 cells) and a GEM-X 3' chip (n= 8 channels for a total of 59,295 cells) and were processed on a Chromium X instrument, followed by library preparation, sequencing, and data analysis. (Leukocytes were maintained at room temperature post preparation as placing them on ice may result in granulocyte lysis.) The aggregated UMAP plot on the left highlights the major cell-type populations in this fresh human leukocyte sample, including neutrophils (the large purple cluster). **B.** Both chemistries captured a similar number of cells of each major immune cell type, with the exception of the neutrophil population. The GEM-X Universal 3' Gene Expression v4 assay detected nearly quadruple the number of neutrophils as the Next GEM Universal 3' Gene Expression v3.1 assay.

Applications

Universal 3' Gene Expression assays are tissue- and species-agnostic, allowing for its use in numerous applications in both healthy and diseased samples. GEM-X Universal 3' Gene Expression (v4) will continue to support applications demonstrated with previous versions of the assay. Among its many applications, the technology has been used to:

- Gain single cell insights in a variety of tissues and species, including birds (11), pigs (16), and plants (17)
- Create a comprehensive atlas of all cell types in heterogeneous tissues, including tumors (3), brains from people with Alzheimer's disease (4, 5), and human embryos (10)
- Reveal the cellular drivers of biological processes in healthy and diseased tissues as they impact early development (10, 11), aging (9), and anti-tumor immune responses (12)
- Uncover cell types and cell states associated with diseases like glioblastoma (1) and lupus (7) to inform drug development, biomarker discovery, and patient stratification
- Delineate the cell populations driving therapeutic efficacy and resistance to treatments, such as small-molecule inhibitors (2) and chemotherapy (13) in cancer patients and epidural electrical stimulation in people with paralysis (6)
- Conduct a multicenter scRNA-seq study on bone marrow samples from 18 international longitudinal study participants with rapidly progressing or non-progressing multiple myeloma (14)
- Identify novel cell populations that impact the immune response to vaccinations and infectious diseases, such as COVID-19, using a combination of single cell gene expression and cell surface protein data (8)

Justification for using GEM-X Universal 3' Gene Expression for your research

GEM-X Universal 3' Gene Expression offers many advantages, making it an optimal product for single cell transcriptomics and multiomics. These include:

- **Simple and robust workflow**—GEM-X Universal 3' Gene Expression enables gene expression or multiomic profiling at single cell resolution using our tried-and-tested Chromium workflow.
- **Demonstrated technology**—Chromium assays have been used as a cornerstone technology in many peer-reviewed papers in high-caliber journals, including *Science*, *Cell*, *Nature Neuroscience*, *Nature Communications*, and *Nature Protocols*.
- **High reproducibility and sensitivity**—Inter- and intra-chip reproducibility experiments demonstrate a UMI correlation score of >0.95 across multiple time points.
- **Multiomic capabilities**—Combined with Feature Barcode technology, GEM-X Universal 3' Gene Expression enables simultaneous detection of tens to hundreds of cell surface proteins alongside gene expression profiling.
- **Optimized conditions for diverse samples**—GEM-X Universal 3' Gene Expression is tissue- and species-agnostic, as demonstrated with whole cells, including cell lines, primary cells, and cryopreserved cell suspensions, as well as nuclei.
- **Comprehensive data analysis solution**—GEM-X Universal 3' 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 transcriptomic profiles, cell clustering, and differential expression analysis for genes and cell surface proteins.
- **Broad support resources**—10x Genomics provides comprehensive support resources, ranging from technical specialists trained in Universal 3' Gene Expression workflows to freely available videos and documents that guide users through the workflow.
- **Certified Service Providers**—Get streamlined access to the complete Universal 3' Gene Expression workflow through Certified Service Providers, third-party facilities specially trained and verified by 10x Genomics to support a wide variety of single cell biology research applications.
- **Certified product quality**—10x Genomics product development and manufacturing processes are ISO 9001:2015 certified.

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Publications

The utility of all Chromium Single Cell assays is demonstrated in numerous peer-reviewed publications, many in top journals. Visit 10xgenomics.com/resources/publications to see the most current list of publications.

Resources

- Single Cell Buyer's Guide
- GEM-X Universal 3' Gene Expression Product Sheet
- Single Cell Analysis
- Software tutorials, documentation, and more
- 10x Genomics Cloud Analysis (check for availability in your region)
- Chromium GEM-X Single Cell 3' v4 Gene Expression User Guide
- Chromium GEM-X Single Cell 3' v4 Gene Expression with Feature Barcoding technology for Cell Surface Protein User Guide
- Chromium X Series Instrument Brochure

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LIT000223 - Rev B - Grant application resources for Chromium GEM-X Single Cell Gene Expression