Grant application resources for Chromium Single Cell Gene Expression

Summary statement

Complex biological systems result from the coordinated function of individual cells, each playing a unique part that contributes to the whole. Because of this complexity, gene expression research in organisms, tissues, or cell populations is often limited by traditional bulk RNA-seq methods that provide an average view of the transcriptional landscape, masking sample heterogeneity and unique cell populations. To address this limitation, 10x Genomics has developed single cell RNA-sequencing methods that enable analysis of transcriptomes on a cell-by-cell basis through the use of microfluidic partitioning to capture single cells and prepare barcoded, next-generation sequencing libraries. Single cell transcriptional profiling can reveal the cellular diversity of a complex sample, with direct application to discovering novel cell types and states, untangling the cellular processes driving disease and development, and understanding the mechanisms that determine response or resistance to therapeutic treatments. To reach even higher levels of insight, single cell RNA-seq data can be combined with the simultaneous detection of additional analytes, including cell surface proteins for defining cell states.

Sample size and throughput needs will vary according to experimental design and will grow along with research goals as the scale and scope of projects increase. Chromium Single Cell Gene Expression provides maximum flexibility for throughput capacity, leveraging the same tried-and-true workflow and chemistry to enable seamless transitions from pilot studies to million-cell experiments. The option to choose the appropriate scale while working within the same system provides necessary consistency when making key transitions throughout various stages of research.

Chromium Single Cell Gene Expression workflow

Chromium Single Cell Gene Expression provides mRNA profiling at single cell resolution. The workflow begins with a suspension of single cells or nuclei. For gene expression analysis alone, unlabeled cells or nuclei can be used.

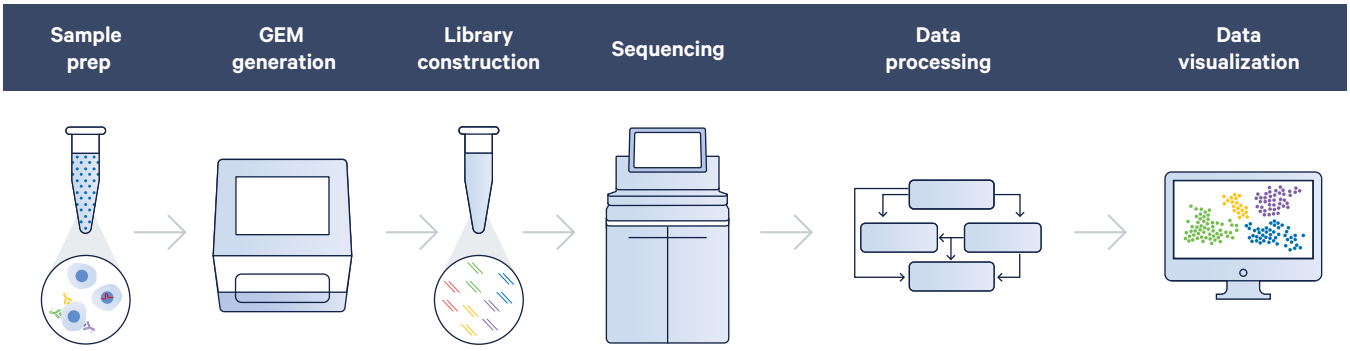
Transcriptomes are analyzed on a cell-by-cell basis through the use of microfluidic partitioning to capture single cells and prepare barcoded, next-generation sequencing (NGS) libraries. Specifically, single cells, reverse transcription (RT) reagents, Gel Beads containing barcoded oligonucleotides, and partitioning oil are combined on a microfluidic chip to form nanoliter-scale reaction vesicles. Within each reaction vesicle, a single cell is lysed, the Gel Bead is dissolved to free the identically barcoded RT oligonucleotides into solution, and reverse transcription of polyadenylated mRNA occurs. As a result, all cDNAs from a single cell will have the same barcode, allowing the sequencing reads to be mapped back to their single cells of origin. The preparation of NGS libraries from these barcoded cDNAs is then carried out in a highly efficient bulk reaction.

Chromium Single Cell Multiomics workflow

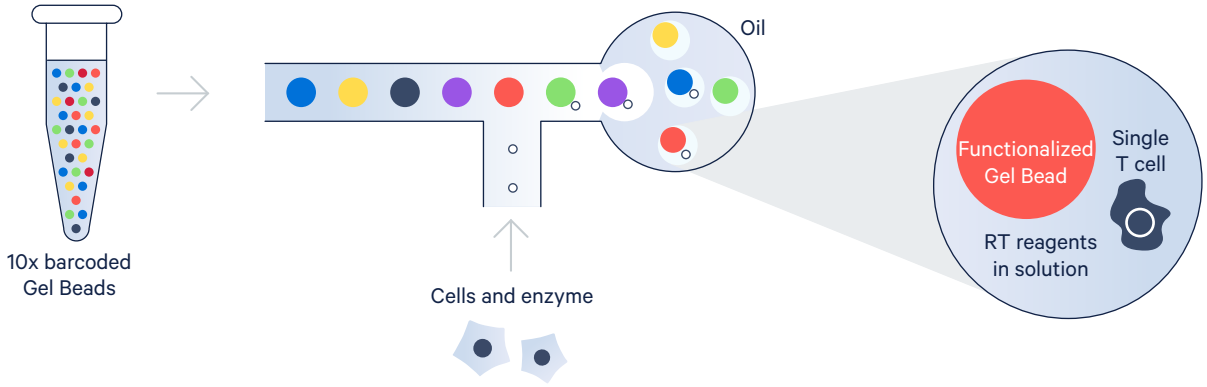
Chromium Single Cell assays also provide the opportunity for single cell multiomic phenotyping with the ability to simultaneously profile hundreds of cell surface proteins along with 3' gene expression analysis. In the above workflow, the single cell suspension used as input would refer to cells labeled with oligo-conjugated antibodies. The workflow would then proceed along the same lines as the standalone gene expression assay, but with the addition of Feature Barcode technology, extension of antibody-conjugated DNA barcodes occurs within the reaction vesicles.

Analysis of combined transcriptome and proteome data sets for individual cells allows for enhanced characterization of cell subtypes and states. The addition of protein information effectively increases the resolution of transcriptionally similar cell subtypes, allowing for the discovery of novel cell subtypes and rare cell subpopulations, as well as help in refining definitions of cell types and functional states.

Workflow overview

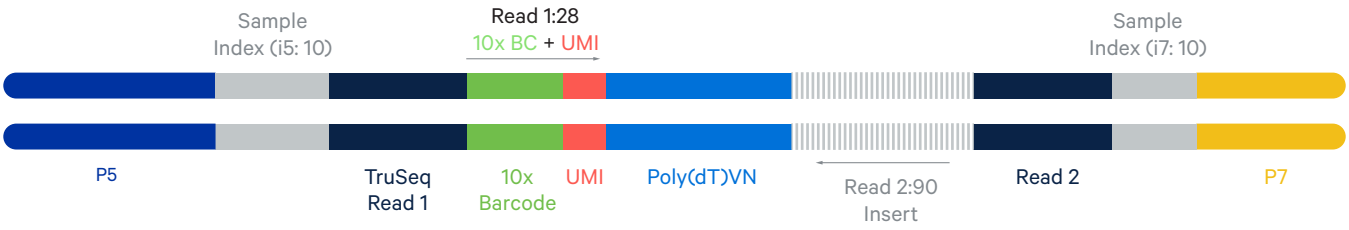


Single cell workflow for gene expression. The workflow begins with a suspension of unlabeled single cells or nuclei, or cells labeled with oligo-conjugated antibodies. Following GEM generation, up to two libraries can be constructed from a single sample, including gene expression and cell surface protein, generating multiple readouts that can be linked back to the same single cell. After sequencing, data are processed with Cell Ranger and sample heterogeneity can be visualized with Loupe Browser, our fully integrated and easy-to-use analysis and visualization software tools.

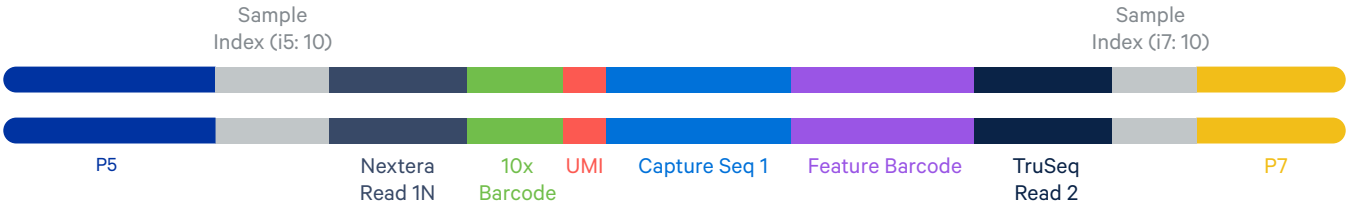


Next GEM technology and single cell partitioning. Using advanced microfluidics, Chromium Single Cell instruments encapsulate Gel Beads in GEMs, or a "Gel Bead-in-emulsion," along with uniquely barcoded single cells or nuclei and reagents to create a micro-reaction.

Single Cell Gene Expression Libraries



Chromium Single Cell 3' Gene Expression Dual Index Library. A Chromium Single Cell 3' Gene Expression Dual Index library comprises standard Illumina paired-end constructs that begin and end with P5 and P7. The 16-nucleotide 10x Barcode and 12-nucleotide unique molecular identifier (UMI) are encoded in Read 1, while Read 2 is used to sequence the cDNA fragment.



Chromium Single Cell 3' Cell Surface Protein Dual Index Library. A Chromium 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-nucleotide 10x Barcode and 12-nucleotide unique molecular identifier (UMI) are encoded in Read 1, while Read 2 is used to sequence the antibody-conjugated DNA barcode.

Data processing and analysis

Following sequencing, BCL or FASTQ files can be analyzed using the Cell Ranger analysis pipeline and visualized using Loupe Browser. Both software tools are available for download on the 10x Genomics Support website.

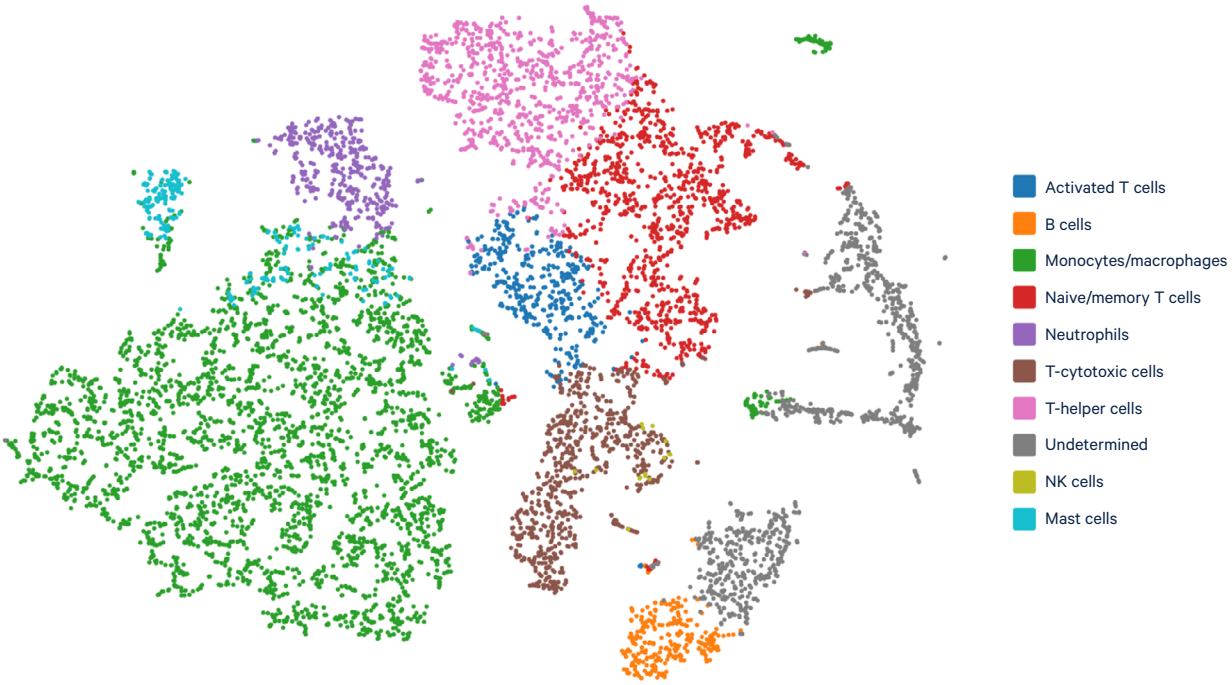
Cell Ranger performs sample demultiplexing, barcode processing, and counting of transcripts and proteins in single cells. Secondary analyses, such as dimensionality reduction, cell clustering, and differential gene expression, are also included. The desktop visualization tool, Loupe Browser, enables interactive data exploration of cell clusters and differential gene expression to aid in data interpretation.

With Loupe Browser, you can:

- Find significant genes—Determine genes that uniquely characterize clusters at the press of a button.
- Identify cell types—Use gene lists and the gene expression view to locate different cell types and functional groups.
- Assess data quality—Compare clustering resolution and performance across samples.

Data benchmarking

In the data highlighted below, approximately 9,000 human peripheral blood mononuclear cells (PBMCs) were processed with Chromium Single Cell Gene Expression. B cells, T cells, T-cell subtypes, monocytes, neutrophils, natural killer (NK) cells, and mast cells could be identified, validating the efficacy of single cell transcriptomic profiling in distinguishing unique immune cell types from a sample.



Characterization and phenotyping of human immune cell types using single cell transcriptomics. Approximately 9,000 human PBMCs were processed using Chromium Single Cell Gene Expression before analysis with Cell Ranger and Loupe Browser. Cell clusters were manually annotated using common marker genes.

Sample and project size considerations

Typically, experimental design and sample preparation for single cell experiments must be determined empirically and may require multiple trials and testing conditions to fully optimize. While standard Single Cell Gene Expression experiments can process thousands to tens of thousands of cells at a time, this level of throughput may not be desirable for pilot data, optimization experiments, or small-scale studies. The Chromium Single Cell Gene Expression LT (Low Throughput) workflow provides an affordable solution for these use cases, offering the high-quality data and reliable analysis of a proven technology with a lower throughput of only 100 to 1,000 cells.

Once experimental conditions are optimized, larger-scale studies can be run with greater confidence using higher throughput methods that allow for increased cell numbers, enabling rare cell type discovery and cell state characterization (1, 4). The Chromium standard and high-throughput (HT) kits offer the ability to efficiently analyze 500–80,000 and 2,000–320,000 cells per run, respectively, without sample multiplexing. As research questions become more complex and samples more diverse, increasing throughput capabilities becomes paramount. The Chromium Single Cell Gene Expression workflow supports 3' CellPlex multiplexing for up to 12 samples, enabling even more ambitious projects with increased cell throughput and a reduced cost per sample.

	Low throughput (LT)	Standard throughput	High throughput (HT)
Example use cases	• Preliminary data for pilot studies or grant applications • Optimization of experimental design or sample preparation • Studies with low cell-throughput needs	• Comprehensive cell or tissue characterization • Rare cell type detection • Analysis of precious samples with limited cell number • Most research applications	• In-depth characterization of numerous complex samples • Analysis of very rare cell types that require higher cell throughput • Multi-sample or time course validation studies
Cell throughput	Efficiently partition 100–1,000 cells (or nuclei) per channel; up to 4 samples per kit	Efficiently partition 500–10,000 cells (or nuclei) per channel without sample multiplexing, or maximize cell throughput even further with 3' CellPlex and recover up to 17,500 singlets* per channel or up to 140,000 singlets per chip	Efficiently partition 2,000–20,000 cells (or nuclei) per channel without sample multiplexing, or maximize cell throughput even further with 3' CellPlex and recover up to 45,000 singlets* per channel or up to 730,000 singlets per chip
Cell capture rate	• Up to 35% loaded cells, with microfluidic doublet rates of 8.0% per 1,000 cells	• Up to 65% loaded cells, with microfluidic doublet rates of 0.8% per 1,000 cells	• Up to 65% loaded cells, with microfluidic doublet rates of 0.4% per 1,000 cells
Multiomic analysis option	Detect cell surface proteins	Detect cell surface proteins or other features	Detect cell surface proteins or other features
Kits	Chromium Single Cell Gene Expression LT, with optional Feature Barcode technology for multiomic analysis	Chromium Single Cell Gene Expression, with optional 3' CellPlex for sample multiplexing or optional Feature Barcode technology for multiomic analysis	Chromium Single Cell Gene Expression HT, with optional 3' CellPlex for sample multiplexing or optional Feature Barcode technology for multiomic analysis
Compatible instruments	Chromium X, Chromium iX, Chromium Controller	Chromium X, Chromium iX, Chromium Connect, Chromium Controller	Chromium X

*Singlets are single cells or nuclei captured after multiplet removal..

Applications

Chromium Single Cell Gene Expression is tissue and species agnostic, allowing for its use in numerous applications in both healthy and diseased samples. Among its many applications, the technology has been used to:

- Create a comprehensive atlas of all cell types in a tissue, such as the human thymus (5).
- Understand the cell type–specific impact of infectious diseases like COVID-19 (6).
- Study how different cell types respond to treatment in cancer and other disease states (7).
- Assess cellular heterogeneity in diseased states, including multiple sclerosis (2).
- Tie cellular phenotypes observed in PBMCs to vaccine responsiveness using a combination of single cell gene expression and cell surface protein data types (3).

Chromium Single Cell Gene Expression advantages

Chromium Single Cell Gene Expression offers many technical advantages, making it an optimal product for single cell transcriptomic profiling. These advantages include:

- Simple and robust workflow—Chromium Single Cell assays leverage proven Next GEM technology to enable gene expression or multiomic profiling at single cell resolution.
- Throughput flexibility—Kits cover the full range of throughput capacity, allowing seamless transitions from small-scale pilot experiments (100s to 1,000s of cells) to large-scale, multi-sample projects (million-cell experiments).
- Optimized conditions for diverse samples—Demonstrated with whole cells, including cell lines, primary cells, cryopreserved cell suspensions, and dissociated fresh and flash frozen tissue, as well as nuclei. Leverage the additional benefit of cell size flexibility, with no lower limits.
- Multiomic capabilities—Combined with Feature Barcode technology, Chromium Single Cell Gene Expression enables simultaneous gene expression profiling and cell surface protein detection for tens to hundreds of antibodies.
- Comprehensive data analysis solution—Chromium Single Cell 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 Chromium Single Cell Gene Expression workflows 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.

References

1. Ding J, et al. Characterization of CD4+ T-cell subtypes using single cell RNA sequencing and the impact of cell number and sequencing depth. *Sci Rep* 10: 19825 (2020).
2. Jäkel S, et al. Altered human oligodendrocyte heterogeneity in multiple sclerosis. *Nature* 566: 543–547 (2019).
3. Kotliarov Y, et al. Broad immune activation underlies shared set point signatures for vaccine responsiveness in healthy individuals and disease activity in patients with lupus. *Nat Med* 26: 618–629 (2020).
4. Pandey S, et al. Comprehensive identification and spatial mapping of habenular neuronal types using single-cell RNA-seq. *Curr Biol* 28: 1052–1065.e7 (2018).
5. Park JE, et al. A cell atlas of human thymic development defines T cell repertoire formation. *Science* 367: eaay3224 (2020).
6. Ren X, et al. COVID-19 immune features revealed by a large-scale single-cell transcriptome atlas. *Cell* 184: 1895–1913.e19 (2021).
7. Stewart CA, et al. Single-cell analyses reveal increased intratumoral heterogeneity after the onset of therapy resistance in small-cell lung cancer. *Nat Cancer* 1: 423–436 (2020).

Additional Resources

- Single Cell Buyer's Guide
- Experiment Planning Guide: Getting started with single cell gene expression
- Software Tutorials
- 10x Genomics Cloud Analysis (United States only)
- Single Cell Gene Expression LT User Guides
- Single Cell Gene Expression User Guides
- Single Cell Gene Expression HT User Guides

Resources from 10x Genomics

We are dedicated to helping you get the most out of your 10x Genomics system by offering multiple helpful resources:

Technology brochure
Discover the power of single cell partitioning and learn how Next GEM technology enables integrated analysis of single cells at massive scale.

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