

Grant application resources for Chromium Single Cell Gene Expression LT

Summary

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.

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 standard throughput methods that allow for greater cell numbers, enabling rare cell type discovery and cell state characterization ([Ding et al. 2020](#), [Pandey et al. 2018](#), see also the [Single Cell 3' LT v3.1 Technical Note](#)).

Chromium Single Cell Gene Expression LT

Chromium Single Cell Gene Expression LT provides mRNA profiling at the single cell level for 100–1,000 cells per sample. The workflow begins with a suspension of single cells or nuclei. For gene expression analysis alone, unlabeled cells or nuclei can be used. If performing single cell multiomic analysis by simultaneous gene expression profiling and cell surface protein detection, oligo-conjugated antibody-labeled cells are used as input.

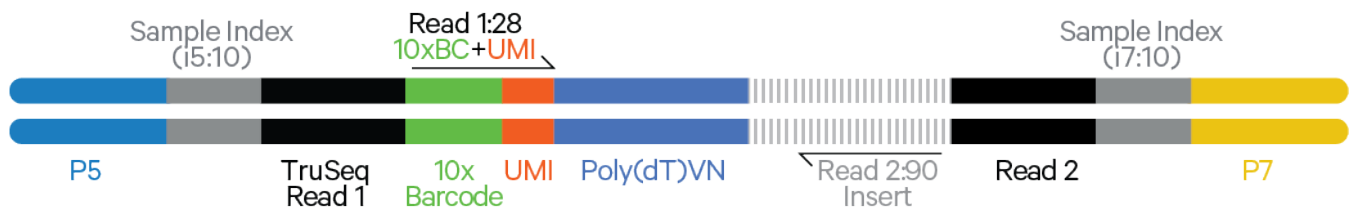
Transcriptomes and, when applicable, cell surface protein expression, are analyzed on a cell-by-cell basis through the use of microfluidic partitioning to capture single cells and prepare barcoded, next-generation sequencing (NGS) libraries. Specifically, single cells, reverse transcription (RT) reagents, Gel Beads containing barcoded oligonucleotides, and 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, along with extension of antibody-conjugated DNA barcodes, if applicable. 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.

Workflow



Single cell workflow for gene expression. The workflow begins with a suspension of unlabeled single cells or nuclei, or oligo-conjugated antibody-labeled cells. 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.

Single Cell Gene Expression Library



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.

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.

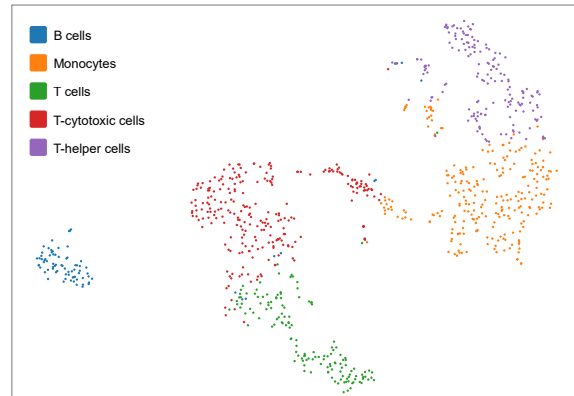
Cell Ranger performs sample demultiplexing, barcode processing, and counting of transcripts and proteins in single cells. Secondary analyses, such as dimensionality reduction, cell clustering, and differential gene expression, are also included. The desktop visualization tool, Loupe Browser, enables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

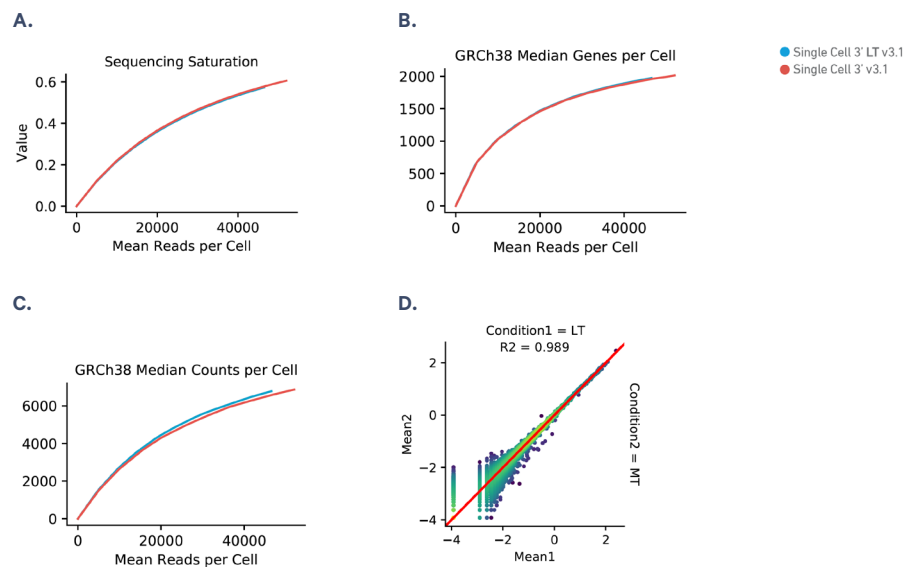
Data benchmarking

In the data highlighted below, approximately 1,000 human peripheral blood mononuclear cells (PBMCs) were processed with Chromium Single Cell Gene Expression LT. B cells, T cells, T-cell subtypes, and monocytes could be identified, validating the efficacy of low-throughput single cell transcriptomic profiling in distinguishing common cell types.



Common cell types can be identified using low-throughput single cell transcriptomics. Approximately 1,000 human PBMCs were processed using Chromium Single Cell Gene Expression LT before analysis with Cell Ranger and Loupe Browser. Cell clusters were manually annotated using common marker genes.

Gene expression profiles were compared from approximately 1,000 PBMCs recovered using Chromium Single Cell Gene Expression LT and approximately 9,000 PBMCs recovered using standard Chromium Single Cell Gene Expression. Both assays resulted in comparable library complexity, gene expression sensitivity, and biological profiles, as plotted below.



Comparable sensitivity is achieved with low-throughput single cell gene expression. Human PBMCs were processed using either Single Cell Gene Expression LT (~1,000 cells) or standard Single Cell Gene Expression (~9,000 cells) and show comparable sequencing saturation (A), median genes per cell (B), median UMI counts per cell (C), and UMI correlation (D).

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 (4)
- Understand the cell-type specific impact of infectious diseases like COVID-19 (5)
- Study how different cell types respond to treatment in cancer and other disease states (6)
- Assess cellular heterogeneity in diseased states, including multiple sclerosis (2)

Chromium Single Cell Gene Expression LT advantages

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

- Simple and robust workflow—Efficiently partition 100–1,000 cells per channel, leveraging proven [Next GEM technology](#).
- Validated results—Recover gene expression profiles for up to 35% of loaded cells for a high-sensitivity solution with microfluidic doublet rates of ~8.0% per 1,000 cells.
- 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 LT 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 LT 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 the Chromium Single Cell Gene Expression LT 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.

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. 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.
4. Park JE, et al. A cell atlas of human thymic development defines T cell repertoire formation. *Science* 367: eaay3224, 2020.
5. Ren X, et al. COVID-19 immune features revealed by a large-scale single-cell transcriptome atlas. *Cell* 184: 1895–1913.e19, 2021.
6. 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](#)
- [Chromium Next GEM Single Cell 3' LT v3.1: Reagents, Workflow & Data Overview Technical Note](#)

Contact us

[10xgenomics.com](https://www.10xgenomics.com) | info@10xgenomics.com

© 2021 10x Genomics, Inc. FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
LIT000125 - Rev A - Grant Application Resources - Chromium Single Cell Gene Expression LT

