TECHNICAL NOTE

Explore the transcriptome with single-cell resolution

- Measure gene expression across tens of thousands of single cells using Chromium Single Cell Gene Expression from 10x Genomics
- Sequence 3' gene expression libraries using the NovaSeq™ 6000, NextSeq™ 2000, NextSeq 1000, or NextSeq 550 systems
- Analyze how cellular heterogeneity contributes to complex biological systems with easy-to-use software

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Introduction

Single-cell sequencing is a next-generation sequencing (NGS) method that examines the transcriptomes of individual cells, providing a high-resolution view of cell-to-cell variation. Traditional RNA sequencing (RNA-Seq), where tissues are sampled in bulk, provides an average readout across cell populations. In contrast, single-cell RNA sequencing (scRNA-Seq) methods enable researchers to tease apart cellular heterogeneity in complex samples. Single-cell data can help researchers discover novel cell populations, identify new regulatory pathways, and reconstruct lineage relationships to understand factors that contribute to tissue development, function, and disease states.[1,2]

This technical note outlines a protocol for scRNA-Seq using Chromium Single Cell Gene Expression from 10x Genomics on Illumina platforms. This method uses oligonucleotide barcodes to directly measure 3' gene expression at the single-cell level for hundreds to tens of thousands of cells.

Protocol overview

This scRNA-Seq experiment follows a workflow of sample preparation, single-cell partitioning and library prep, sequencing, and analysis (Figure 1). The protocol leverages the Chromium platform and Next GEM technology from 10x Genomics and proven Illumina sequencing technology. Beginning with a single-cell or single-nuclei suspension, the Chromium instrument and reagents isolate single cells in droplets that contain a barcoded gel bead.

Reverse transcription and amplification generate a barcoded sequencing-ready single-cell gene expression library. Libraries are sequenced on an Illumina production-scale sequencing platform, such as the NovaSeq 6000, NextSeq 2000, NextSeq 1000, or NextSeq 550 system. Data analysis with the Cell Ranger pipeline (10x Genomics) maps gene expression results to each individual cell with the help of barcodes from the gel beads. Loupe Browser software (10x Genomics) makes it easy to visualize and explore single-cell gene expression data and characterize the heterogeneity of the sample. Single-cell sequencing data can also be analyzed using the Illumina DRAGEN™ Single-Cell app, available on BaseSpace™ Sequence Hub in the cloud and locally on the NextSeq 1000 and NextSeq 2000 instruments.

Prepare samples

Sample input for the Chromium Single Cell Gene Expression protocols should be a single-cell (or single-nuclei) suspension that is free of excess debris. The assay has been validated on various human and mouse tissue types, and monkey and rat cell types.[3] Researchers have demonstrated compatibility with other species as well.[4,5] Proper sample handling and preparation techniques preserve the integrity of the cellular membrane and are critical for generating high-quality single-cell data.[3]

Cell preparation guide,
10x Genomics Support website

Demonstrated protocols,
10x Genomics Support website

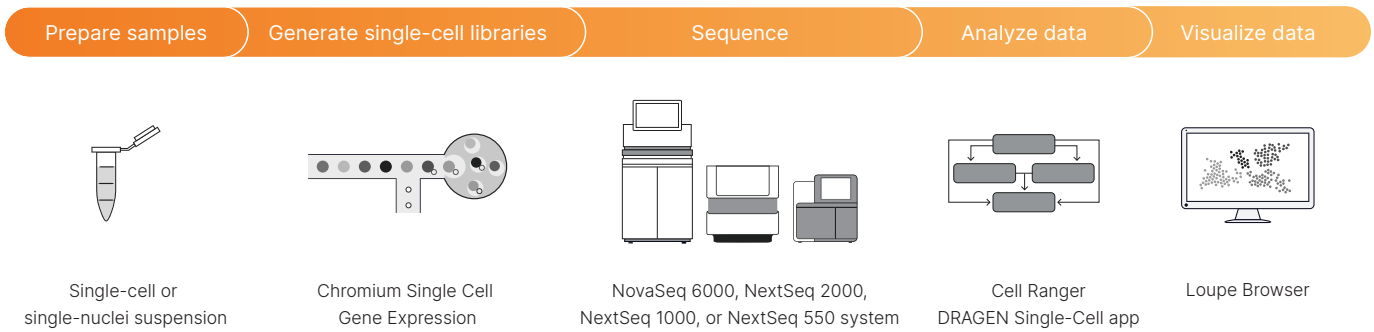

Figure 1: Workflow for a single-cell gene expression experiment—Start with a single-cell or single-nuclei suspension, followed by microfluidic single-cell partitioning and barcoding with the Chromium instrument. Sequence the resulting single-cell gene expression library on Illumina systems. Analyze and visualize data using Cell Ranger and Loupe Browser software or the DRAGEN Single-Cell pipeline.

Generate single-cell libraries

Prepared single-cell suspensions are ready for library preparation using a Chromium Next GEM Single Cell Gene Expression kit (Figure 2). Use the LT kit for low-throughput pilot studies (100-1000 cells), standard kit for cell numbers up to 80,000, and HT kit for high-throughput studies (up to 320,000 cells).*[6-8] Cell suspensions are loaded onto a microfluidic chip and run on the Chromium instrument, which partitions individual cells into droplets, each with a single gel bead that contains a unique barcode.

Within each droplet, or "GEM" (gel bead-in-emulsion), the gel bead captures the 3' polyA tails of the cell's mRNA and reverse transcription enzymes add barcodes to the first-strand cDNA. Following reverse transcription, GEMs are broken and pooled fractions are recovered and purified. Library construction steps generate barcoded single-cell gene expression libraries, ready for sequencing on Illumina sequencing systems.

Sequence with Illumina instruments

To accommodate the sequencing output required for this application, we recommend sequencing the scRNA-Seq libraries on the NovaSeq 6000, NextSeq 2000, NextSeq 1000, or NextSeq 550 system (Table 1). Smaller scale instruments like the iSeq™ 100 System can be used to optimize experimental design.[9]

The NovaSeq 6000, NextSeq 2000, and NextSeq 1000 systems offer additional features for cost-efficient scRNA-Seq experiments.[10] The 100-cycle reagent kits (available for NextSeq 1000/2000 P2, NextSeq 2000 P3, and NovaSeq SP, S1, and S2 flow cells) are compatible with Chromium Single Cell Gene Expression libraries. The NovaSeq 6000, NextSeq 2000, and NextSeq 1000 systems also offer a run planner on BaseSpace Sequence Hub for easy setup and demultiplexing of the run.

Single cell RNA product compatibility, Illumina Support website

Chromium Single Cell Gene Expression dual index libraries comprise standard Illumina paired-end constructs that are flanked with i5 and i7 indexes used for demultiplexing (Table 2, Figure 3). Sequence single-cell gene expression libraries using a paired-end sequencing run.

10x Genomics recommends a minimum of 20,000 read pairs per cell for the Chromium Single Cell Gene Expression assay (Table 1). Adjust sequencing depth for the required performance or application. For the sequencing run setup, 10x Genomics recommends a PhiX library spike-in of at least 1%. PhiX acts as a control for the run and provides information regarding error rate metrics. Example sequencing metrics for single-cell gene expression libraries are available on the 10x Genomics Support website[11] and Illumina BaseSpace Sequence Hub demo data page.[12]

* The Chromium Single Cell Gene Expression standard kit requires 500 cells minimum; HT kit requires 2000 cells minimum.

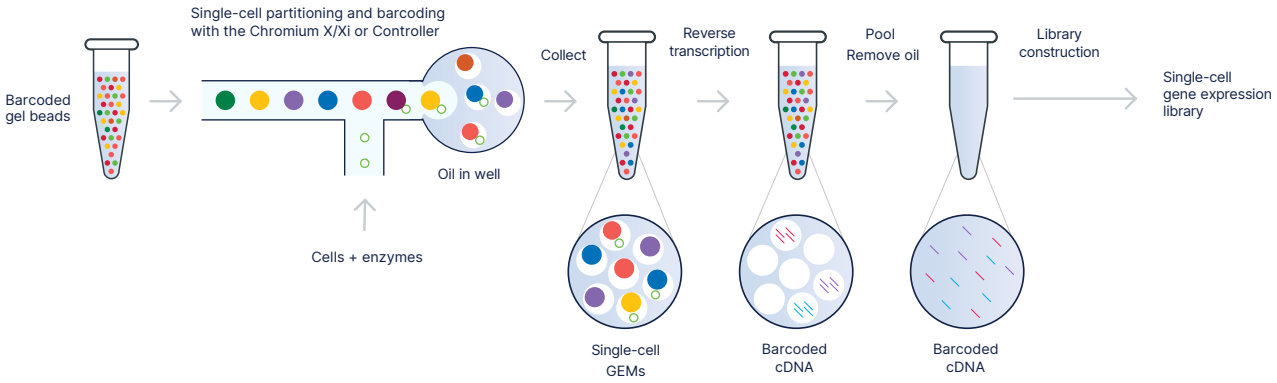

Figure 2: Generate 3' gene expression libraries from individual cells—Single cells are captured in GEMs, where the 3' ends of mRNA are barcoded. GEMs are broken and pooled before cleanup, cDNA amplification, and library construction. This generates a library from each sample to link gene expression back to an individual cell.

Table 1: Example sample throughput for Chromium Single Cell Gene Expression on Illumina sequencing systems

Chromium Single Cell Gene Expression library type	Minimum read pairs per cell[b]	Maximum supported cell number per library[c]	No. of samples per run[a]							
			NextSeq 550	NextSeq 2000			NovaSeq 6000			
			High output	P1[d]	P2[d]	P3	SP	S1	S2	S4
Low throughput	20K	1K	20	5	20	60	32	65	165[e]	384[e]
Standard	20K	10K	2	-	2	6	3	6	16	40
High throughput	20K	20K	1	-	1	3	1	3	8	20

a. The number of single-cell samples per sequencing run is based on an Illumina PhiX control library at supported cluster densities and loading concentration; actual performance parameters may vary based on sample type, sample quality, and clusters passing filter; see the specification sheet for each sequencing system for more details
b. Minimum read recommendations provided courtesy of 10x Genomics; 20K read pairs per cell or 40K individual reads, 20K from Read 1 and 20K from Read 2; adjust sequencing depth for the required performance or application; the sequencing saturation metric and curve in the Cell Ranger run summary can be used to optimize sequencing depth for specific sample types
c. Maximum supported cell number per library assumes no sample multiplexing is performed during the Chromium workflow; sample multiplexing using the 3' CellPlex product from 10x Genomics allows for scaling up cell throughput per library
d. P1 and P2 flow cells with the same sample throughput also available on the NextSeq 1000 System
e. With NovaSeq XP workflow only, which allows for individual lane loading on NovaSeq flow cells; a maximum of 384 unique dual indexes is available

Table 2: Recommended sequencing read lengths for Chromium Single Cell Gene Expression libraries

Library type	Read 1	i7 index	i5 index	Read 2
Purpose	Cell barcode and UMI	Sample index	Sample index	cDNA insert
Dual index[a] 3' gene expression libraries	28 bp	10 bp	10 bp	90 bp
Single index 3' gene expression libraries	28 bp[b]	8 bp	0 bp	91 bp[b]

a. The dual index configuration is recommended for new users to mitigate index hopping
b. Shorter transcript reads may lead to reduced transcriptome alignment rates. Cell barcode, unique molecular identifier (UMI), and sample index reads must not be shorter than indicated; any read can be longer than recommended; Cell Ranger will automatically ignore any additional bases in cell barcode or UMI reads

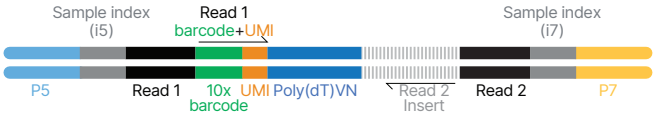

Figure 3: Configuration of single-cell dual index gene expression libraries ready for sequencing—Read 1 primer is used for sequencing the 28-bp cell barcode and unique molecular identifier (UMI). Read 2 primer is used for sequencing the cDNA insert.

Analyze and visualize your data

Following sequencing, the Cell Ranger analysis pipelines will align reads and count barcodes and UMIs to map transcripts in single cells. Cell Ranger software identifies clusters of cells with similar gene expression profiles to turn raw sequencing data into results. Cell Ranger can also aggregate outputs from multiple experiments, normalize to the same sequencing depth, and reanalyze the combined data. The primary output of Cell Ranger is a count matrix, consisting of columns for every cell barcode and rows for all measured features, such as genes for transcriptome analysis. The analysis pipeline output includes quality-control information[13] and files that can be exported for further analysis in Loupe Browser visualization software or third-party R or Python tools.

Illumina DRAGEN Bio-IT Platform (available in the cloud, on-premise server, or onboard the NextSeq 1000 and NextSeq 2000 Sequencing Systems) also offers tools for scRNA-Seq data analysis. The DRAGEN Single-Cell pipeline outputs quality-control metrics and a cell-by-gene expression matrix that is compatible with popular single-cell analysis tools like Scanpy, AnnData, and Seurat.

Data highlights

Single-cell transcriptomic analysis enables deep characterization of cell types and states at low throughput (Figure 4) or high throughput (Figure 5).

Sample data sets and run outputs, BaseSpace Sequence Hub public data page

Access expert support

For sequencing Chromium Single Cell Gene Expression libraries, the Illumina and 10x Genomics teams collaborate to ensure you are fully supported throughout the workflow. Contact 10x Genomics Support (support@10xgenomics.com) for assay and analysis questions, and Illumina Support (techsupport@illumina.com) for sequencing questions. The teams are also equipped to handle more complex issues together.

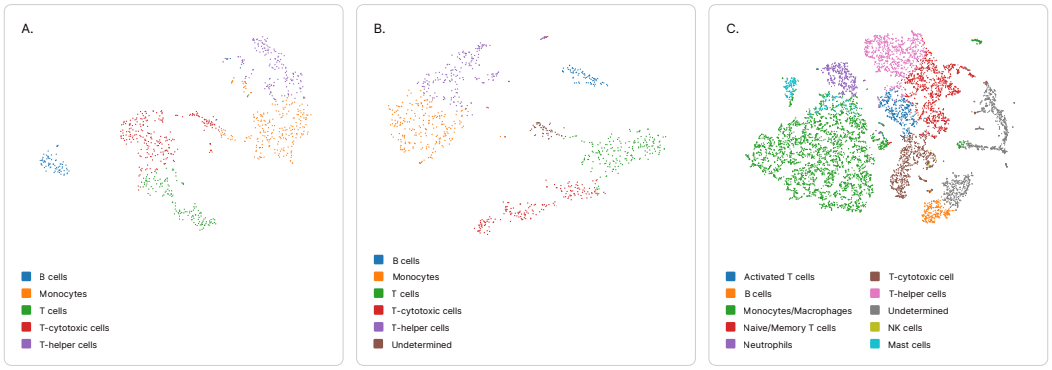

Figure 4: Single-cell gene expression in PBMCs data highlight—Unsupervised clustering and manual annotation of gene expression data derived from human peripheral blood mononuclear cells (PBMCs) profiled using the (A) Chromium Single Cell Gene Expression LT assay (~900 cells), (B) standard Chromium Single Cell Gene Expression assay (~900 cells), and (C) standard assay (~8500 cells). Cells are clustered based on whole-transcriptome profiles and manually annotated using common gene expression markers. The LT and standard assays produce comparable data resolution at comparable cell loads. Scaling up the number of cells profiled using the standard kit allows for deeper profiling of cell types and states.[6,7]

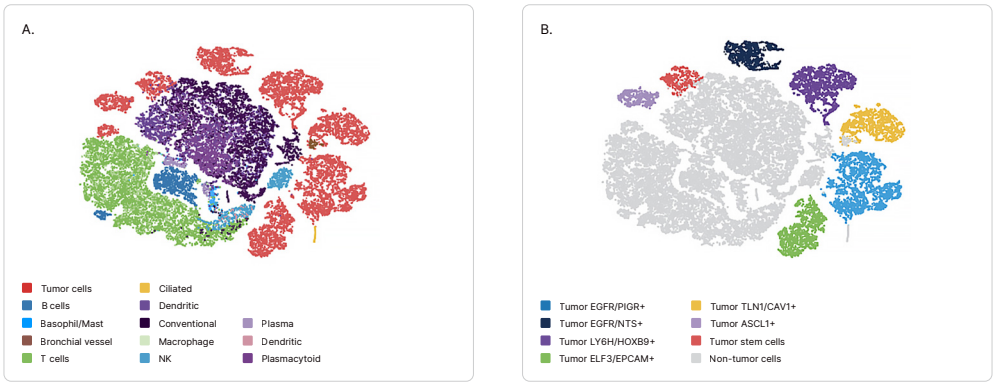

Figure 5: High-throughput single-cell gene expression in tumor cells data highlight—Unsupervised clustering and manual annotation of gene expression data from dissociated human non-small cell lung cancer tumor sample using the Chromium Single Cell Gene Expression HT assay (~12,600 tumor cells and ~25,800 non-tumor cells). (A) Cells are clustered based on whole-transcriptome profiles and manually annotated using common gene expression markers. (B) Tumor cells are classified in more detail based on specific cancer gene markers. The Single Cell Gene Expression HT assay is designed for experiments that require a higher level of throughput, such as ultra-rare cell discovery, high-throughput CRISPR and drug screens, or minimal residual disease (MRD) detection.[8]

Summary

This single-cell gene expression protocol enables transcriptome analysis for dissociated samples with single-cell resolution, providing insights into biological heterogeneity. With the ability to distinguish cell types and reveal dynamic cell states, researchers can gain a deeper understanding of cellular phenotypes and complex systems.

Learn more

Single-cell sequencing, illumina.com/techniques/sequencing/rna-sequencing/ultra-low-input-single-cell-rna-seq.html

NovaSeq 6000 System, illumina.com/systems/sequencing-platforms/novaseq.html

NextSeq 1000 and NextSeq 2000 Systems, illumina.com/systems/sequencing-platforms/nextseq-1000-2000.html

NextSeq 550 System, illumina.com/systems/sequencing-platforms/nextseq.html

Chromium Single Cell Gene Expression, 10xgenomics.com/products/single-cell-gene-expression

References

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12. Illumina. BaseSpace Sequence Hub Demo Data. basespace.illumina.com/datacentral. Accessed January 4, 2022.
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Ordering information

Product	Catalog no.
NovaSeq 6000 SP Reagent Kit v1.5 (100 cycles)	20028401
NovaSeq 6000 S1 Reagent Kit v1.5 (100 cycles)	20028319
NovaSeq 6000 S2 Reagent Kit v1.5 (100 cycles)	20028316
NovaSeq 6000 S4 Reagent Kit v1.5 (200 cycles)	20028313
NextSeq 1000/2000 P2 Reagents v3 (100 cycles)	20046811
NextSeq 2000 P3 Reagents (100 cycles)	20040559
NextSeq 500/550 Mid Output Kit v2.5 (150 cycles)	20024904
NextSeq 500/550 High Output Kit v2.5 (150 cycles)	20024907

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