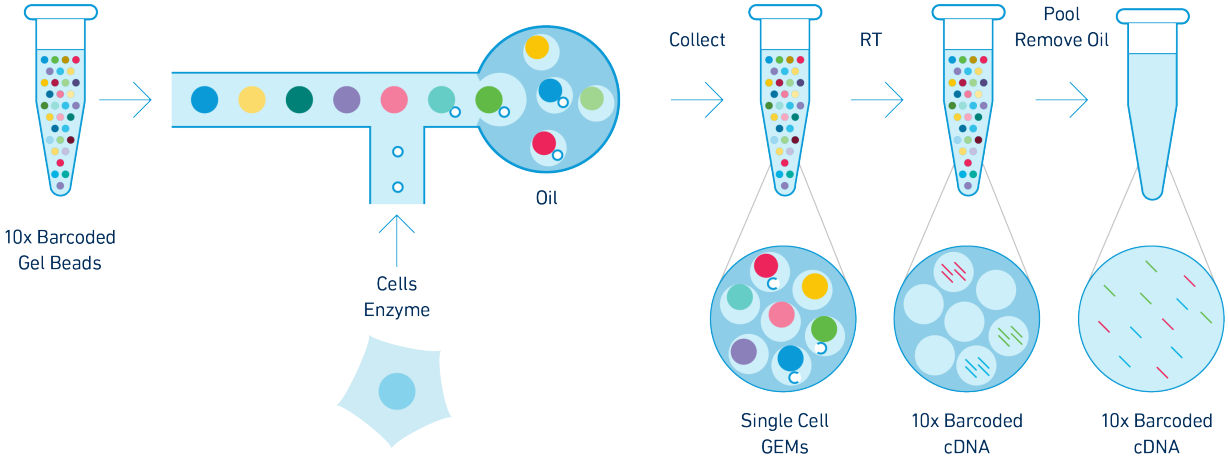
Getting Started: Single Cell 3' Gene Expression

Introduction



The Chromium Single Cell Gene Expression Solution provides a scalable microfluidic platform for gene expression profiling of 500-10,000 individual cells per sample. A pool of ~3,500,000 10x Barcodes are sampled separately to index each cell's transcriptome. It is done by partitioning thousands of cells into nanoliter-scale Gel Beads-in-emulsion (GEMs), where all generated cDNA share a common 10x Barcode. Dual indexed libraries are generated and sequenced from the cDNA and 10x Barcodes are used to associate individual reads back to the individual partitions. To learn more about the chemistry behind the assay, click here.

Sample Preparation Guidelines

Careful sample preparation provides the foundation for successful single cell RNA-seq experiments. For more information, consult Common FAQs on Sample Preparation.

Obtaining a Single Cell Suspension

Fresh tissue must be dissociated into a single cell suspension. For cultured cells or cells already in suspension, a cell washing protocol must be performed to remove media. Cryopreservation or methanol fixation may be used to store cells prior to performing single cell RNA-seq.

Consult the 10x Genomics Single Cell Preparation Guide as well as 10x Genomics Demonstrated Protocols for guidance on sample preparation for various sample types.

For additional tissue-specific resources, including tissue types not tested by 10x Genomics, consult the Worthington Tissue Dissociation Guide. Optimization may be required for the specific tissue type.

Optimal Sample Parameters

Cell Viability	Cell Concentration	Cell Size
>70% Use an automated cell counter or a hemocytometer with a live/dead stain to determine cell viability. In cases of low cell viability, improve viability by removing dead cells.	700-1,200 cells/µl Consult Guidelines for Accurate Target Cell Counts. Measuring cell count is critical for accurate target cell recovery, as detailed in this Technical Note. Refer to the Cell Count table for expected cell recovery rates.	Up to 30 µm Large cells (>30 µm) and hard to dissociate samples may require nuclei isolation. Consult Isolation of Nuclei for Single Cell RNA Sequencing. Small cells or cells with low RNA content may require additional modifications to the assay workflow.

Sample Preparation Guidelines

Cell Counts for Accurate Cell Recovery

The Cell Count table below shows the expected recovery rates for various cell loads.

Multiplet Rate (%)	# of Cells Loaded	# of Cells Recovered
~0.4%	~800	~500
~0.8%	~1,600	~1,000
~1.6%	~3,200	~2,000
~2.3%	~4,800	~3,000
~3.1%	~6,400	~4,000
~3.9%	~8,000	~5,000
~4.6%	~9,600	~6,000
~5.4%	~11,200	~7,000
~6.1%	~12,800	~8,000
~6.9%	~14,400	~9,000
~7.6%	~16,000	~10,000

Materials Required and Step Overview

Consult the user guide for a consumables and equipment list validated by 10x Genomics for executing the Chromium Single Cell 3' Gene Expression protocol.

To enable seamless experimental planning, a breakdown of the protocol steps, along with execution times and stop & store points are listed below.

10x Genomics how-to video series provides a visual demonstration of the workflow.

Day	Steps		Timing	Stop & Store
2 h	**Cell Preparation**			
	Dependent on Cell Type		~1-1.5 h	
	Step 1 – GEM Generation & Barcoding			
	1.1	Prepare Reaction Mix	20 min	
	1.2	Load Chromium Next GEM Chip G	10 min	
4 h	1.3	Run the Chromium Controller	18 min	
	1.4	Transfer GEMs	3 min	
	1.5	GEM-RT Incubation	55 min	STOP 4°C ≤72 h or –20°C ≤1 week
	Step 2 – Post GEM-RT Cleanup & cDNA Amplification			
	2.1	Post GEM RT-Cleanup – Dynabead	45 min	
	2.2	cDNA Amplification	40 min	STOP 4°C ≤72 h or –20°C ≤1 week
6 h	2.3	cDNA Cleanup – SPRIselect	20 min	STOP 4°C ≤72 h –20°C ≤4 weeks
	2.4	cDNA QC & Quantification	50 min	
	Step 3 – 3' Gene Expression Library Construction			
	3.1	Fragmentation, End Repair & A-tailing	50 min	
	3.2	Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRIselect	30 min	
	3.3	Adaptor Ligation	25 min	
	3.4	Post Ligation Cleanup- SPRIselect	20 min	
8 h	3.5	Sample Index PCR	40 min	STOP 4°C ≤72 h
	3.6	Post Sample Index PCR Double Sided Size Selection- SPRIselect	30 min	STOP 4°C ≤72 h or -20°C long term
	3.7	Post Library Construction QC	50 min	

Feature Barcode Technology

Feature Barcode technology allows for the capture of additional information at the single cell level, including cell surface proteins and directly linked CRISPR perturbations.

For more information, contact BioLegend or Sigma-Aldrich. In the TotalSeq product line, only TotalSeq-B is fully supported with the Chromium Single Cell Gene Expression Solution.

Sequencing

Follow the recommended sequencing run parameters and depth for sequencing Chromium Single Cell 3' Gene Expression libraries.

Single-index libraries

	Read 1	i7 Index	i5 Index	Read 2
Purpose	Cell Barcode & UMI	Sample Index	N/A	Insert
Length (bp)*	28	8	0	91

Dual-index libraries

	Read 1	i7 Index	i5 Index	Read 2
Purpose	Cell Barcode & UMI	Sample Index	Sample Index	Insert
Length (bp)*	28	10	10	90

*Shorter transcript reads may lead to reduced transcriptome alignment rates. Cell barcode, UMI, and Sample Index reads must not be shorter than indicated. Any read can be longer than recommended.

A list of 10x Genomics validated sequencers and expected data throughput can be found in the Sequencing Metrics for Illumina Sequencers Technical Note.

Contact:
support@10xgenomics.com
10x Genomics
6230 Stoneridge Mall Road
Pleasanton, CA 94588 USA