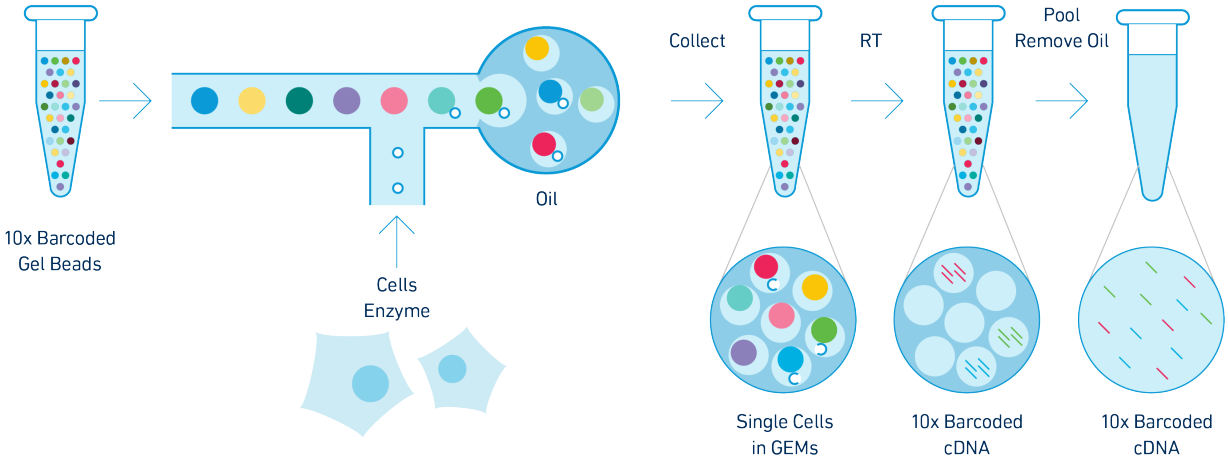
Getting Started: Single Cell Immune Profiling

Introduction



The Chromium Single Cell Immune Profiling solution allows for simultaneous generation of full-length, paired V(D)J sequences, as well as gene expression, cell surface protein expression, and antigen specificity data at a single cell level. The assay partitions thousands of cells into nanoliter-scale Gel Beads-in-emulsion (GEMs), where all generated cDNA within a single droplet share a common 10x Barcode. Libraries are generated and sequenced from the cDNA and the 10x Barcodes are used to associate each read back to its individual partition. 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. The following are tips and best practices for preparing samples for single cell RNA-seq. For more information, consult Common FAQs on Sample Preparation.

Obtaining a Single Cell Suspension

From fresh tissue: fresh tissue must be dissociated into single cell suspension. Consult 10x Genomics Demonstrated Protocols for guidance on sample preparation for various sample types (frozen, fixed, tumors). Optimization may be required for the specific tissue type.

From cultured cells or cells already in suspension: a cell washing protocol should be performed to remove media. Consult the 10x Genomics Single Cell Preparation Guide for details.

Immune cell enrichment: depending on sample type, consider enriching the sample for immune cells prior to running the assay.

For additional tissue specific resources, including tissue types not tested by 10x Genomics, please consult the Worthington Tissue Dissociation Guide.

Optimal Sample Parameters

Cell Viability: >70%	Cell Concentration: 700-1,200 cells/µl	Cell Size: up to 30 µm
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.	Use an automated cell counter or a hemocytometer with a live/dead stain to determine cell viability 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 table below for expected cell recovery rates.	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 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

Equipment and consumables required are outlined in the introduction of each user guide. The key steps of the Single Cell Immune Profiling Protocol are highlighted in the figure below.

	Steps	Timing	Stop & Store
3 h	**Cell Preparation and Labeling** Dependent on cell type and labeling protocol used	~1-2 h	
	Step 1 – GEM Generation & Barcoding		
	1.1 Prepare Reaction Mix	20 min	
	1.2 Load Chromium Next GEM Chip G	10 min	
	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
6 h	**Step 2 – Post GEM RT Cleanup & cDNA Amplification**		
	2.1 Post GEM-RT Cleanup – Dynabead	45 min	
	2.2 cDNA Amplification	50 min	STOP 4°C ≤72 h or –20°C ≤1 week
	2.3 cDNA Cleanup	15 min	STOP 4°C ≤72 h or –20°C ≤1 week
	2.4 cDNA Quantification & QC	50 min	
	*After cDNA Amplification & QC, for V(D)J Amplification and V(D)J Library Construction proceed to steps 3-4. For 5' Gene Expression Library Construction proceed directly to step 5.		
8 h plus* *Time dependent on Stop options used and protocol steps executed	**Step 3 – V(D)J Amplification from cDNA**		
	3.1 V(D)J Amplification 1	40 min	STOP 4°C ≤72 h
	3.2 Post V(D)J Amplification 1 Double Sided Size Selection –	20 min	STOP 4°C ≤72 h or –20°C ≤1 week
	3.3 SPRIselect	40 min	STOP 4°C ≤72 h
	3.4 V(D)J Amplification 2	30 min	STOP 4°C ≤72 h or –20°C ≤1 week
	3.5 Post V(D)J Amplification 2 Double Sided Size Selection – SPRIselect	50 min	
	Step 4 – V(D)J Library Construction		
	4.1 Fragmentation, End Repair & A-tailing	45 min	
	4.2 Adaptor Ligation	25 min	
	4.3 Post Ligation Cleanup – SPRIselect	20 min	
	4.4 Sample Index PCR	40 min	STOP 4°C ≤72 h
	4.5 Post Sample Index PCR Cleanup – SPRIselect	20 min	STOP 4°C ≤72 h or –20°C long-term
	4.6 Post Library Construction QC	50 min	
	Step 5 – 5' Gene Expression (GEX) Library Construction		
	5.1 GEX Fragmentation, End Repair & A-tailing	45 min	
	5.2 GEX Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRIselect	30 min	
	5.3 GEX Adaptor Ligation	25 min	
	5.4 GEX Post Ligation Cleanup – SPRIselect	20 min	
	5.5 GEX Sample Index PCR	40 min	STOP 4°C ≤72 h
	5.6 GEX Post Sample Index PCR Double Sided Cleanup – SPRIselect	30 min	STOP 4°C ≤72 h or –20°C long-term
	5.7 GEX Post Library Construction QC	50 min	

A visual demonstration of the workflow is available in the 10x Genomics how-to video series.

Feature Barcode Technology

Feature Barcode technology allows for the capture of additional information at the single cell level, including cell surface proteins and antigen specificity. Contact BioLegend or Immudex for more information on these reagents. For the TotalSeq product line, only TotalSeq-C is compatible with the Chromium Single Cell Immune Profiling Solution.

Sequencing

Follow the sequencing requirements for single cell V(D)J libraries for read lengths and sequencing depth.

Single-index libraries.

Read	Read 1	i7 Index	i5 Index	Read 2
Purpose	10x Barcode & UMI	Sample Index	N/A	Insert
Length**	26	8	0	91

Dual-index libraries.

Read	Read 1	i7 Index	i5 Index	Read 2
Purpose	10x Barcode & UMI	Sample Index	N/A	Insert
Length**	26	10	10	90

For gene expression libraries, shorter transcript read lengths may lead to reduced alignment rates. For V(D)J libraries, shorter transcript read lengths may lead to a decrease in the number of cells with productive V-J spanning pairs. Read lengths less than 77 bp are not supported for V(D)J libraries. 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.

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