

Single cell profiling on more samples with no time constraints

Overview

Single cell RNA sequencing is increasingly being used to profile larger numbers of samples, corresponding to cohorts of patients or different perturbations, making an efficient and scalable workflow of paramount importance. Fixation with paraformaldehyde (PFA) allows samples to be collected, shipped to a central location, and analyzed without sacrificing integrity or data quality, creating new possibilities for sample accessibility, throughput, and batched analysis. Multiomic capabilities allow for more thorough characterization of cell populations with high-throughput capture of cell surface protein alongside gene expression. Advanced chemistry also brings single cell transcriptomic profiling to FFPE tissue, expanding the range of accessible sample types. Profile up to a million fixed single cells at once with a scalable workflow that can be run on your schedule, while accommodating the needs of translational and clinical labs where fragile samples or time constraints would otherwise preclude analysis.

Highlights

- Fix fresh samples at the point of collection to lock in biological states and preserve fragile cells
- Improve sample collection, preparation, and processing steps to streamline single cell workflows
- Reduce experimental variability and increase efficiency by batching and multiplexing samples
- Generate large-scale and highly sensitive measurements of gene expression and cell surface proteins simultaneously
- Expand single cell possibilities with access to archived samples

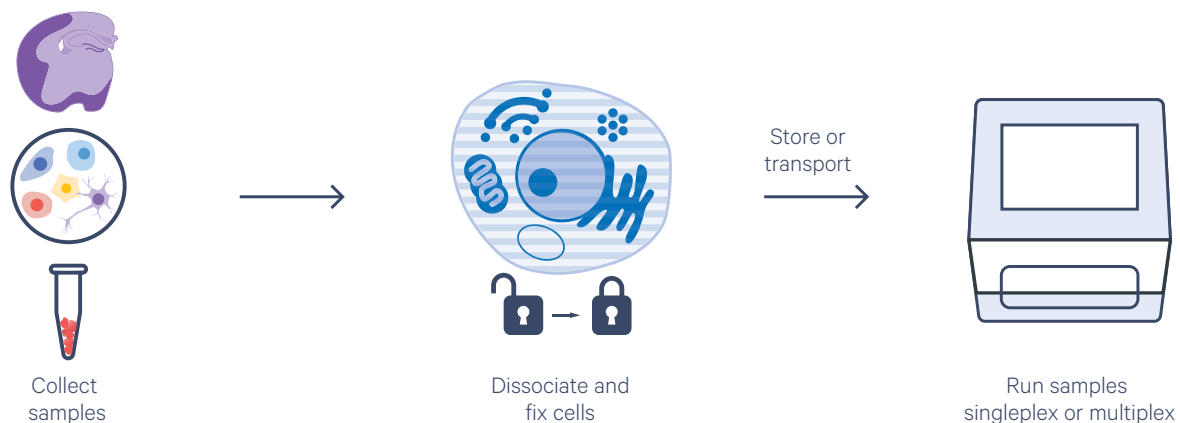


Figure 1. Gene expression profiling on your schedule. Chromium Single Cell Fixed RNA Profiling enables single cell gene expression studies on precious samples that were previously inaccessible because of logistical challenges in sample handling. Fixation at the point of sample collection preserves fragile biology and greatly streamlines workflows to provide critical insights on more samples.

Product features

- Profile gene expression for thousands to hundreds of thousands of cells or nuclei with a sensitive probe-based method that captures the whole human or mouse transcriptome to detect even low-expressing genes
- Store samples without losing data quality, allowing you to batch samples in the same run and minimize experimental variability
- Choose your scale, with the flexibility to run 1–128 samples or up to 1 million cells per run with multiplexing
- Lock in cell state and then combine gene expression analysis with detection of cell surface proteins at single cell resolution for ultra-high parameter multiomic cytometry, using singleplex or multiplex workflows
- Follow a ready-to-use, robust workflow with optimized demonstrated protocols for diverse sample types, including cell lines, primary cells, and dissociated tissue containing fragile cells
- Bring single cell profiling to FFPE tissue from clinical trials or other translational studies

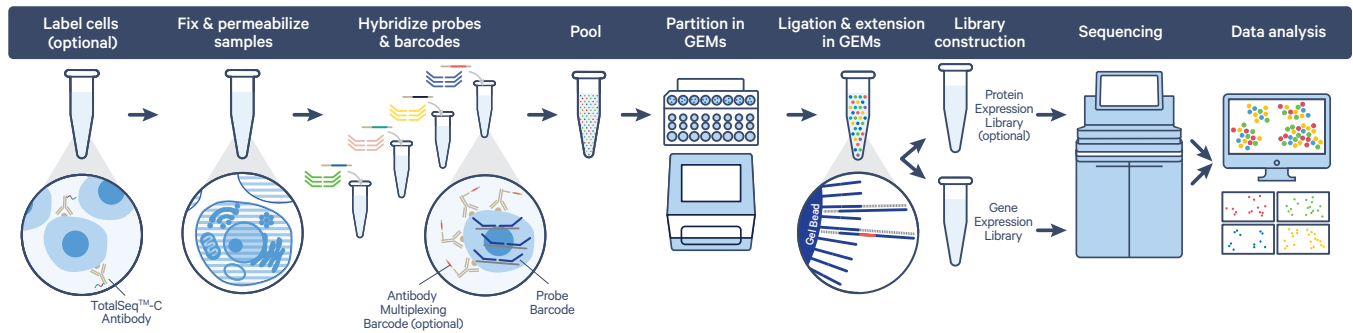


Figure 2. Single Cell Gene Expression Flex enables an efficient and streamlined workflow for sample management and processing. Prior to sample fixation, cells can be labeled with antibody-oligonucleotide conjugates to enable cell surface protein expression (optional) alongside gene expression. Fixed cells are then permeabilized and can be safely stored or transported without compromising data quality. Once ready to proceed, samples are hybridized to probe sets and may be processed individually (singleplex workflow) or pooled with up to 16 samples in a single lane of a Chromium chip (multiplex workflow). During GEM generation, the probe sets are ligated and extended to incorporate unique barcodes. Sequencing libraries are then prepared, sequenced, and analyzed using 10x Genomics Cell Ranger and Loupe Browser software tools.

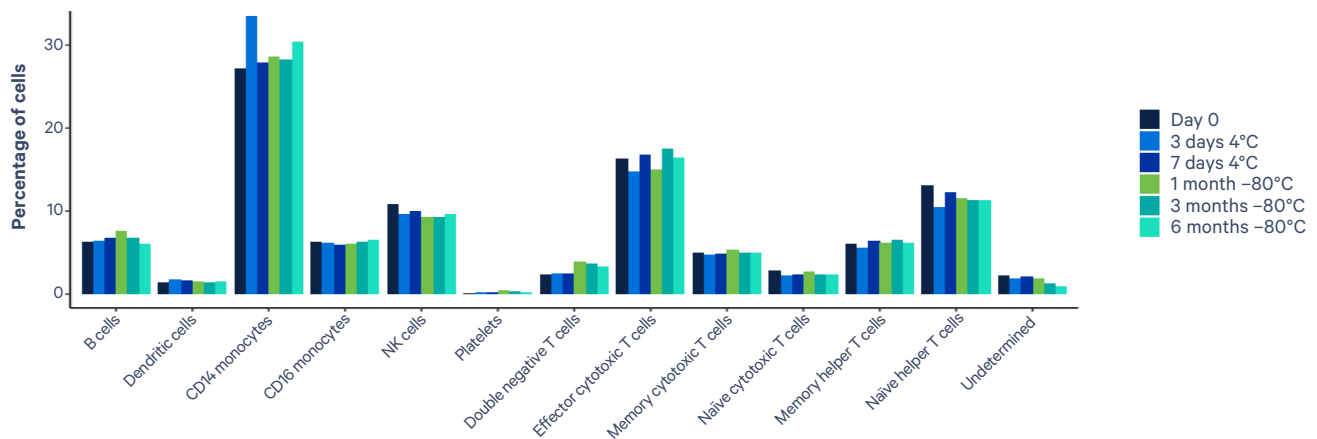


Figure 3. Storage stability of fixed samples. Single cell gene expression profiling of human PBMCs, fixed and run immediately (Day 0); after storage at 4°C for three days or one week; or after storage at -80°C for one, three, or six months. Key populations of cell types are stable over time, demonstrating that the biology can be locked in with PFA fixation to enable transportation and storage, relieving constraints of running single cell experiments.

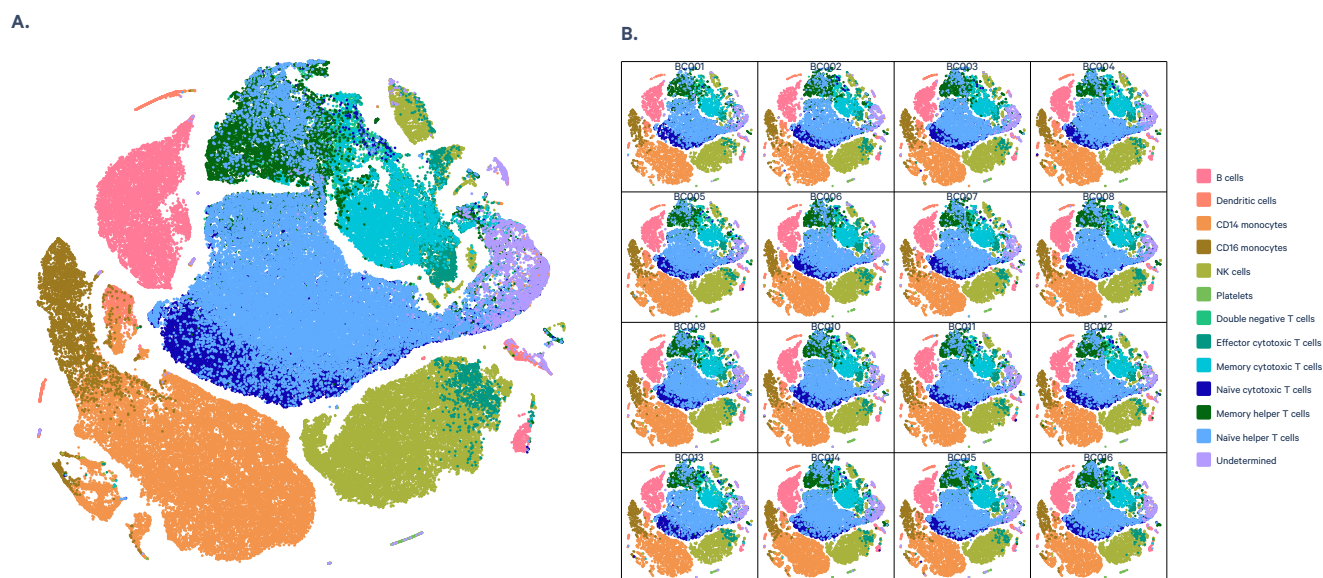
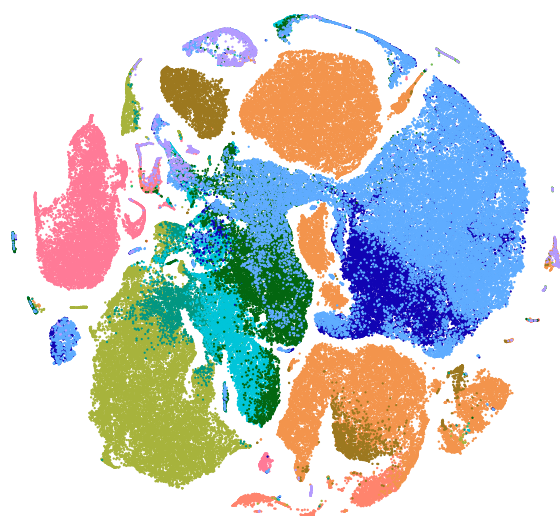


Figure 4. Cell clustering of gene expression data from fixed samples. Human PBMCs were fixed and split evenly into 16 separate hybridization reactions, each with a barcoded set of probes. After hybridization, samples were pooled together in equal proportions and run on a single lane of a Chromium X chip (128,000 cells targeted). **A.** t-SNE plot showing ~119,000 cells from all pools, overlaid together, with distinct clusters representing each cell type. **B.** t-SNE plots showing the same data split by barcode to demonstrate that there were no batch effects and that multiplexing is an effective strategy for reducing experimental variability while vastly increasing sample throughput for single cell experiments.

A. Gene expression



B. Antibody

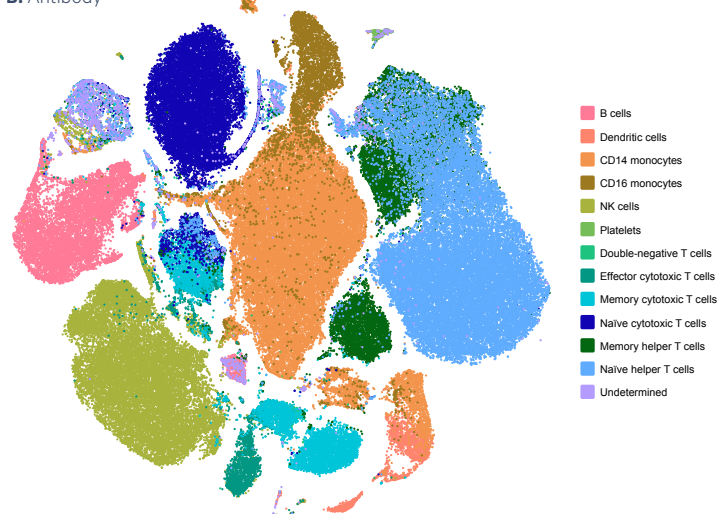


Figure 5. Multiomic characterization of multiplexed samples. Human PBMCs were stained with TotalSeq™-C Human Universal Cocktail V1.0, fixed, and hybridized with barcoded probes and antibody multiplexing barcodes. After hybridization, samples were run on a single lane of a Chromium X chip (128,000 cells targeted). **A.** Gene expression t-SNE plot showing ~140,000 total cells with clusters representing each cell type. **B.** Antibody t-SNE plot of the same PBMC sample. Inclusion of cell surface protein data enabled increased resolution of T-cell subpopulations.

Product specifications

- Singleplex samples: Efficiently partition 500–10,000 cells per channel and run up to 8 samples and 80,000 cells per chip
- Multiplex samples: Multiplex up to 16 samples and 128,000 cells per channel; Run 1–128 samples or up to 1 million cells in one run
- Cell size flexibility, no lower limits
- Compatible with fresh or PFA-fixed tissues, cells, and nuclei, as well as FFPE samples
- Cell surface protein expression is compatible with whole cells, using singleplex or multiplex workflows
- High cell capture rates of ~65%
- Low rates of undetected multiplets at 0.8% per 1,000 cells, with up to 8% at max load per sample

Sample preparation products	Product code
Chromium Next GEM Single Cell Fixed RNA Sample Preparation Kit, 16 rxns	1000414
Chromium Nuclei Isolation Kit with RNase Inhibitor (optional)	1000494
Gene expression profiling products	Product code
Chromium Fixed RNA Kit, Human Transcriptome, 4 rxns x 1 BC	1000474
Chromium Fixed RNA Kit, Human Transcriptome, 4 rxns x 4 BC	1000475
Chromium Fixed RNA Kit, Human Transcriptome, 4 rxns x 16 BC	1000476
Chromium Fixed RNA Kit, Human Transcriptome, 16 rxns x 16 BC	1000547
Chromium Fixed RNA Kit, Mouse Transcriptome, 4 rxns x 1 BC	1000495
Chromium Fixed RNA Kit, Mouse Transcriptome, 4 rxns x 4 BC	1000496
Chromium Fixed RNA Kit, Mouse Transcriptome, 4 rxns x 16 BC	1000497
Chromium Fixed RNA Kit, Mouse Transcriptome, 16 rxns x 16 BC	1000568
Chromium Next GEM Chip Q Single Cell Kit, 16 rxns	1000422
Chromium Next GEM Chip Q Single Cell Kit, 48 rxns	1000418
Dual Index Kit TS Set A, 96 rxns	1000251
Feature Barcode technology	Product code
Fixed RNA Feature Barcode Kit, 16 rxns	1000419
Dual Index Kit TT Set A, 96 rxns	1000215
Fixed RNA Feature Barcode Multiplexing Kit, 64 rxns	1000628
Dual Index Kit TN Set A, 96 rxns	1000250
Instrument compatibility	Product code
Chromium iX & Accessory Kit, 12 Mo. Warranty	1000328
Chromium iX & Accessory Kit, 24 Mo. Warranty	1000329
Chromium X & Accessory Kit, 12 Mo. Warranty	1000331
Chromium X & Accessory Kit, 24 Mo. Warranty	1000332
Software	
Cell Ranger	Download Cloud analysis
Loupe Browser	Download
Compatible partner products	
Biolegend TotalSeq™-B Biolegend TotalSeq™-C www.biolegend.com/totalseq	Learn more

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10xgenomics.com | info@10xgenomics.com

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