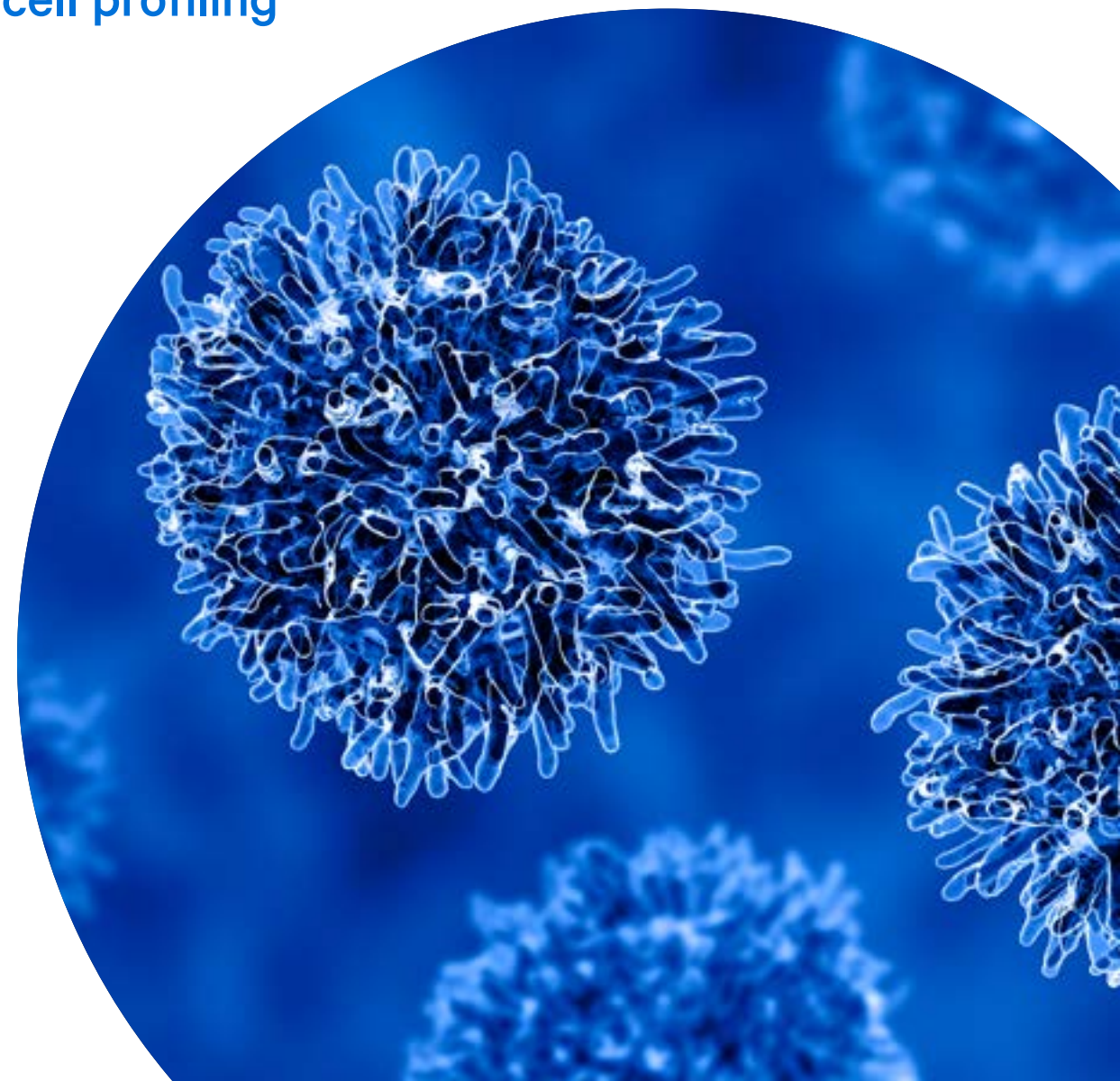
10x GENOMICS

Flex Apex Grant Application Resource

Unmatched performance and scale for single cell profiling



10xgenomics.com

Flex Apex

Summary statement

Each of the assays in the Chromium single cell platform are developed to meet specific research needs and deliver high-performance, reproducible results for users of varying experience. Here, we discuss the unique benefits of **Chromium GEM-X Flex Apex (GEM-X Flex v2)**, which leads our single cell portfolio with unmatched sensitivity and scale, and is powered by optimized probe-based chemistry and plate-based sample handling. Our Chromium single cell assays have already transformed how researchers approach some of the most critical questions in human health and disease. The Apex assay and its Flex technology take that foundation even further, unlocking greater scope, scale, and impact to enable groundbreaking discoveries in oncology, neuroscience, immunology, developmental biology, and drug discovery/development.

With Apex, multiple high-performance features come together to enable more powerful studies: our proven GEM-X microfluidics provide robust results you can count on across experiments, while quick and easy sample fixation preserves biology at the point of collection to allow sample storage and batching without compromising sample quality. Furthermore, 96-well multiplexing brings a streamlined workflow and automation compatibility to studies of any scale, with on-demand reagent flexibility and the ability to profile up to 100M cells or 384 samples a week. Same-cell multiomics enable deeper insights into complex biology with capture of intracellular and cell surface protein alongside gene expression, plus the ability to run genome-wide, pooled CRISPR screens at single cell resolution.

Chromium platform overview

The Chromium platform from 10x Genomics offers a suite of products enabling analysis of transcriptomes, cell surface and intracellular proteins, paired B- and T-cell receptor sequences, and chromatin accessibility on a cell-by-cell basis. Researchers have used these trusted, reliable assays to significantly advance scientific knowledge, publishing over 9,500 publications to date. These publications cover a breadth of fields in diverse species with high-quality analysis of a wide range of sample types, including fresh, frozen, and fixed samples (even FFPE and fixed whole blood).

10x Genomics is committed to making our robust Chromium assays accessible to researchers with different skill sets through advanced and reliable instrumentation, intuitive software analysis solutions, expert technical support, and trusted partnerships with service providers. These assays are supported by instruments that automate the most critical stages in every protocol: cell partitioning and barcoding. Efficient, reliable automation of this step ensures researchers with varied experience can obtain accurate, reproducible results. Those looking to automate additional steps across the workflow have access to reagents compatible with liquid handling platforms and vetted partnerships with automation experts.

Moving beyond the physical workflows, researchers with or without bioinformatics experience can use our suite of analysis tools and pipelines (including Cell Ranger and Loupe Browser) to process, visualize, and explore their data. Researchers also have access to 10x Genomics technical experts who can provide support through scientific and technical consultations, workflow optimization, and methodology troubleshooting. Researchers in need of further support can take advantage of a global network of Contract Research Organizations (CROs) and Certified Service Providers (CSPs) who can assist with every stage of our workflow—from sample preparation to library generation—and the specific needs of drug discovery researchers, including global sample acquisition and management.

Chromium GEM-X Flex Apex overview

Why single cell resolution matters

Single cell RNA sequencing (scRNA-seq) is a well-established, reliable method that has enabled researchers to delve into the biological complexities driven by cellular heterogeneity in a way other methods—including bulk RNA-seq, flow cytometry, and mass spectrometry—cannot.

For example, traditional bulk RNA-seq provides the average gene expression in multicellular tissue, leaving researchers to infer the proportion of known cell types, cell-to-cell variability in gene expression, and more. Rare cell states and populations are often masked in these datasets. Additionally, traditional methods to analyze intracellular and cell surface protein levels often fail to capture these unique cell populations, especially transient cell states (such as differentiating cells) where genes are undergoing rapid and dynamic regulation before entering a stable cell state with well-defined protein markers. scRNA-seq has allowed researchers to identify these short-lived cell states, leading to key discoveries in early human development (**1**) and more.

End-to-end solutions for robust, high-performance single cell

To address these limitations, 10x Genomics developed our Chromium single cell platform. Now powered by GEM-X technology and supported by the Chromium X series instrument, this end-to-end solution enables researchers to profile tens of thousands of genes at single cell resolution for unbiased characterization of cell types and states. Unique multiomic options allow for simultaneous detection of hundreds of proteins for ultra-high paramter multiomic cytometry, and/or genome-wide CRISPR screens for functional genomics studies (without the need for custom probes).

Apex takes single cell profiling even further, with unique attributes that improve data quality, even for the most challenging samples. Optimized probe-based chemistry delivers our highest gene sensitivity with protein-coding gene coverage for human, mouse, or rat samples. To accommodate additional experimental needs, the pre-designed whole transcriptome panels can be supplemented with your own custom probes for transcripts of interest, including other species, pathogen detection, SNVs, noncoding RNAs, and reporters/custom transgenes. Expert probe design (including 3-fold coverage for most targets) maximizes specificity, sensitivity, and sequencing efficiency, all of which translate to more accurate transcript detection (even for low-expressing genes), greater detection of rare cell types, and greater savings on sequencing budgets. Notably, Apex yields high-quality results even from samples with low-quality or damaged RNA, thanks to a small 50-nucleotide (nt) footprint.

Apex workflows that meet the demands of real-world research

This probe-based chemistry has further advantages in expanded sample-type compatibility, unique sample prep options, and highly flexible workflows that put less strain on daily schedules, regardless of the specific sample collection process. Sample-type compatibility brings high-performance single cell analysis to fresh, frozen, and fixed samples (even FFPE sections and fixed whole blood). Quick and easy fixation protocols preserve biology at the point of collection while reducing urgency from sample processing and streamlining experimental logistics. With

the ability to fix samples, store them for years, and batch them in a single run, even multi-site and longitudinal studies are easier to handle. Sample batching also brings several benefits to the bench, including a reduction in batch effects and the ability to access unprecedented experimental scale.

Remote and/or distributed collection of blood samples is further empowered by protocols designed for the realities of translational research. These workflows allow samples to be collected anywhere, even without onsite lab access or skilled intervention at the collection site. While standard sample collection was not built for the demands of large cohorts, longitudinal studies, or multi-site research, these Apex protocols mean blood samples can be easily stabilized on the spot for downstream processing at a central location.

Unprecedented scale for greater cell and sample throughput

Plate-based multiplexing with Apex transforms large studies with a dramatic increase in scale, enabling researchers to profile up to 384 samples or up to 100 million cells a week with a 96-well format. On-demand reagent flexibility allows researchers to scale at their own pace and reduce waste by running partial plates, using only the reagents they need and saving the rest for later. The convenient 96-well format further accelerates the workflow with automation compatibility and minimized handling, thus freeing up time in the lab and improving consistency across experiments.

Apex workflow

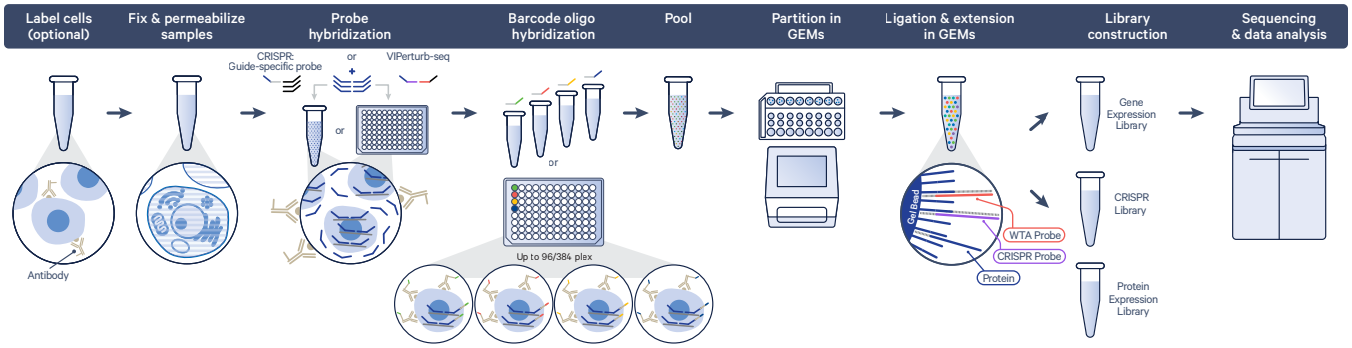


Figure 1. Schematic overview of the Apex technology workflow. A typical workflow starts with user-supplied fresh, frozen, or fixed samples. Before sample fixation, cells can be labeled to enable protein expression alongside gene expression. Cells are then fixed and permeabilized, allowing safe storage without compromising data quality. Once ready to proceed, samples are hybridized to probe sets. CRISPR probe sets can be spiked in at this point for optional perturbation screens. Samples may be processed individually (singleplex workflow) or pooled with up to 384 samples in a single lane of a Chromium chip (plate-based multiplex workflow). The Chromium X instrument facilitates cell partitioning and barcoding for millions of cells in just minutes. During GEM generation, probe sets are ligated and extended to incorporate unique barcodes. Sequencing-ready libraries are then prepared, sequenced, and analyzed using 10x Genomics Cell Ranger pipelines and visualized with the Loupe Browser software.

With Apex, transcriptomes 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 (**Figure 1**). The unique workflow starts with quick and easy sample fixation after collection, enabling safe storage and transport without affecting RNA integrity or data quality. When ready, fixed samples are permeabilized and mixed with the gene expression panel and other GEM-X reagents that enable partitioning, lysis, and barcoding of cellular information.

These master mixes of samples and reagents are loaded onto a microfluidic chip along with Gel Beads and partitioning oil. Gel Beads are coated with oligonucleotides, which include a 10x Barcode that marks each RNA molecule's cell of origin; a unique molecular identifier (UMI) that gives each transcript a unique fingerprint; sequences for capturing transcripts (and protein, if desired); and adapter sequences for downstream next-generation sequencing (**Figure 2**).

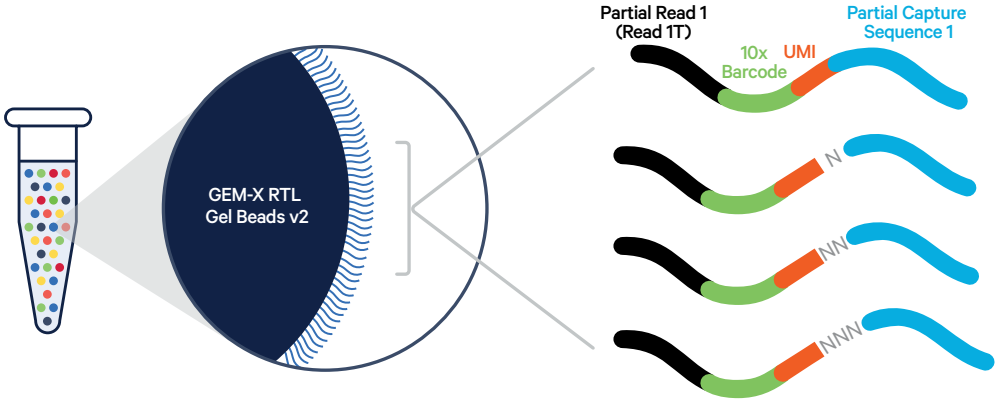


Figure 2. Schematic diagram of a GEM-X Flex Apex Gel Bead. Every Gel Bead is coated with primers containing a partial TruSeq Read 1 (partial Read 1 sequencing primer, partial Read 1T), a 16-nucleotide (nt) 10x GEM Barcode (or 10x Barcode), a 12-nt unique molecular identifier (UMI), and a partial Capture Sequence 1 (sequence complementary to the probe and Antibody Multiplexing Barcode oligonucleotide, if applicable). The primers also contain between 0 and 3 Ns to increase base diversity in the Sample Barcode region when reading from the R1 direction. Note that this example applies to our 16- and 96-sample kits; the Gel Beads for the 4-sample kits contain only the first primer shown in the figure.

Once a loaded microfluidic chip is placed in the Chromium X series instrument, cells move through the channels at a limiting dilution to generate nanoliter-sized reaction vessels known as Gel Beads in Emulsion (GEMs; **Figure 3**). Immediately following GEM generation, the Gel Bead is dissolved, releasing the barcoded Gel Bead primers, and the cell is lysed, releasing the transcripts. An incubation step allows the creation of barcoded fragments that will become the inputs for library generation.

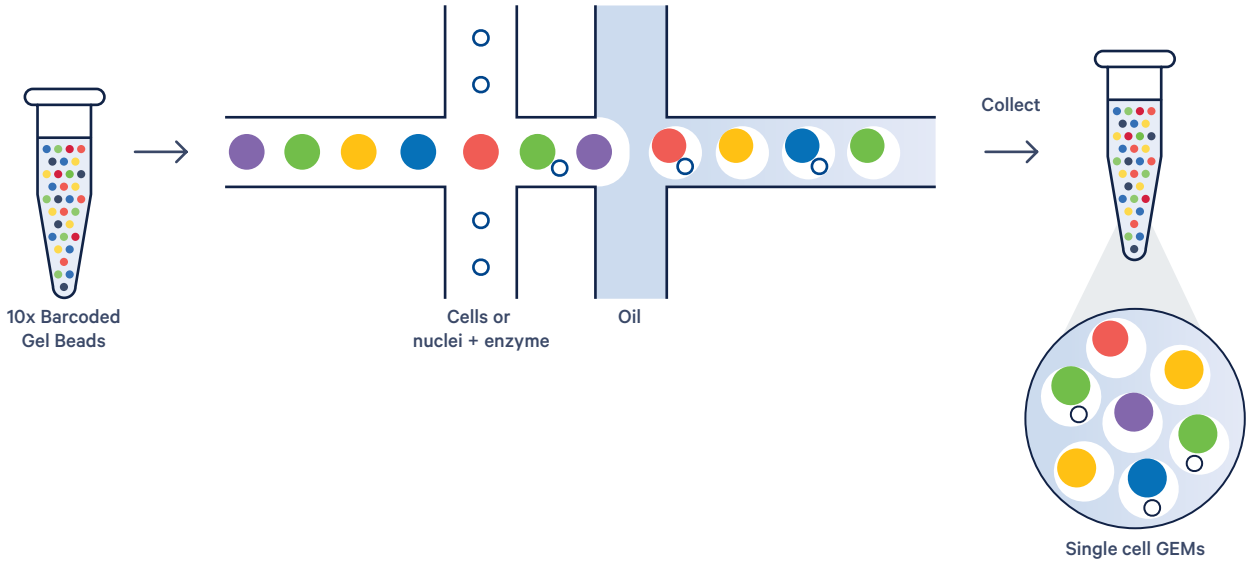


Figure 3. A schematic overview of GEM generation and barcoding with the GEM-X chip workflow. GEMs are generated by combining barcoded Gel Beads, a master mix containing cells and enzymes, and partitioning oil in a GEM-X FX Chip. Cells or nuclei within the GEMs can be identified with unique barcodes, enabling researchers to trace barcoded transcripts back to the cell of origin.

All fragments from a single cell, originating from a single GEM, will have the same 10x Barcode, allowing the user to map each transcript back to its cell of origin. The individual oligos on a single Gel Bead, however, have different UMIs, which distinctly mark each DNA molecule with a unique, random 12-base sequence. This allows the user to eliminate any PCR duplicates during downstream processing, helping ensure more accurate quantitation of transcripts. The barcoded DNA is then used to prepare a sequencing library, which includes the addition of sequencing adapters for PCR amplification. Finally, the library is sequenced with NGS.

Advanced GEM-X technology

GEM-X technology offers optimized reagents, improved microfluidics, and upgraded analysis software to drive improvements to every step of the tried-and-tested Apex workflow. Specifically, GEM generation and cell partitioning are more efficient than with previous iterations of this assay that were powered by Next GEM technology.

With Next GEM technology, GEMs were generated independent of partitioning oil via step emulsification. Now, GEM-X microfluidics utilize the flow of partitioning oil to facilitate GEM formation. With this new process, twice as many GEMs are generated at smaller volumes, reducing multiplet rates two-fold and increasing throughput capabilities. Additionally, cell partitioning and barcoding are faster and more robust, resulting in an abbreviated run time.

The Chromium X series instruments maximize these improvements by automating cell partitioning and barcoding, minimizing the risk of technical error between researchers of varying skill levels. Even researchers conducting large clinical or translational studies across multiple institutions have reported robust results (**2**). Furthermore, advancements to the underlying chemistry of these automated steps in the Apex (GEM-X Flex v2) assay compared to the Next GEM assay translate to key performance improvements:

- Preservation of high sensitivity for our probe-based chemistry, enabling detection of rare transcripts, fragile cells, and cells with low RNA content; notably, Apex demonstrates equivalent or even higher data quality vs. GEM-X Flex v1 (**Figure 4**)
- Higher throughput with a two-fold increase in cells captured per channel (up to 20,000 cells per channel with the singleplex workflow; plate-based multiplexing further increases scale to 1 million cells per channel)
- More cost-effective experiments driven by sample batching, scaling up for higher cell throughput, more efficient use of reagents, and reduced sequencing costs
- Improved sample recovery—up to 80% cell recovery efficiency during cell partitioning, minimized clogging, and maximized cell size flexibility
- Enhanced data quality and robustness due, in part, to half the rate of undetected multiplets (0.4% per 1,000 cells) and more efficient GEM generation
- Faster run times—millions of cells are partitioned and barcoded in just minutes

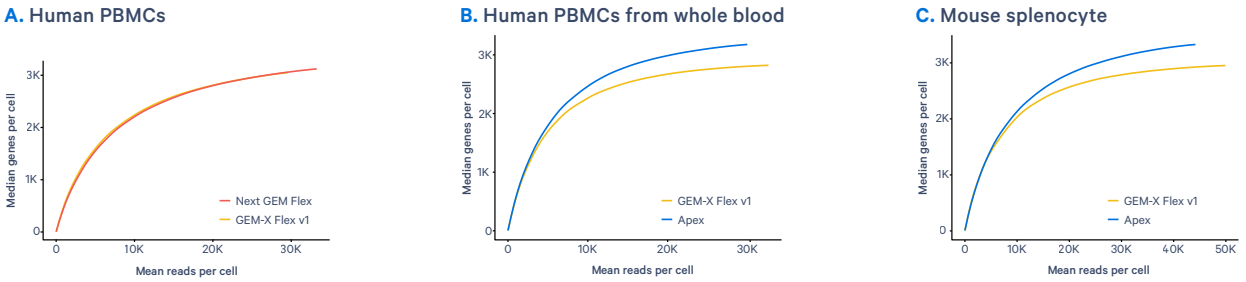


Figure 4. GEM-X technology maintains the high data quality for Flex assays seen with Next GEM kits, while Apex provides equivalent or higher data quality vs. GEM-X Flex v1. A. Human PBMCs were fixed prior to hybridization and processing with GEM-X Flex (v1) and Next GEM Flex workflows. 5,800 cells were loaded for each assay, targeting 4,000 cells each. Samples processed via GEM-X Flex (v1) show comparable gene sensitivity to those processed with Next GEM Flex. **B, C.** Human PBMCs isolated from fixed whole blood and fixed mouse splenocytes were hybridized and processed with each workflow. 5,800 cells were loaded for each assay, targeting 4,000 cells each. Samples processed with Apex (GEM-X Flex v2) show higher gene sensitivity than those processed with GEM-X Flex v1.

Plate-based multiplexing workflow

Plate-based multiplexing with Apex enables game-changing scale for single cell profiling. This unique workflow allows researchers to batch and multiplex samples for their largest studies, with the ability to process up to 384 samples or up to 100M cells per week. Fixed, hybridized samples may be pooled with up to 384 samples in 96-well format. Experiments can be designed to either maximize the number of samples, increase the cell count within a sample (sample subpooling), or use a combination of both strategies. Furthermore, this streamlined workflow minimizes handling and adds automation compatibility, attributes that help ensure high consistency and increased efficiency.

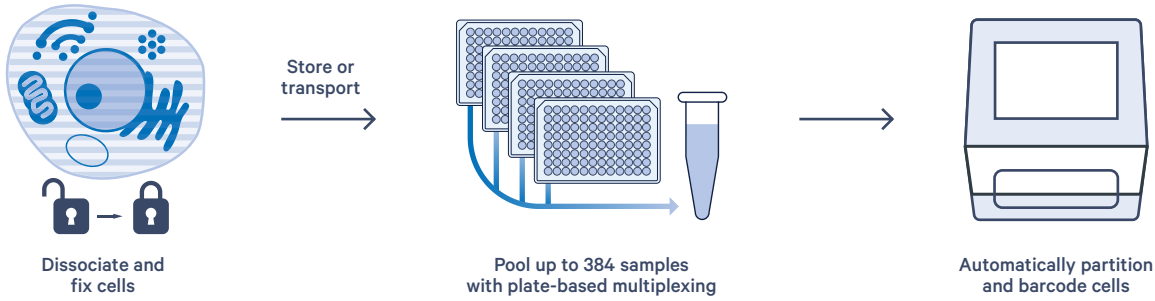


Figure 5. Overview of plate-based multiplexing workflows with Apex. Fixation at the point of sample collection preserves biology and greatly streamlines workflows. Plate-based multiplexing dramatically increases scale and improves sample handling with automation compatibility. Apex enables multiplexing of up to 384 samples through hybridization with barcoding oligos, each containing a specific Sample Barcode. These unique barcode oligos provide experimental flexibility to run arrayed screens, batch samples, or leverage multiple barcodes to screen more cells (see pages 25 and 26 of the User Guide for details).

While the multiplexing option empowers ambitious studies, it also offers the opportunity for researchers to scale at their own pace. On-demand reagent flexibility means partial plates can be run as needed, helping to reduce waste, making sure that each experiment uses only the reagents it needs, and allowing the rest to be saved for later.

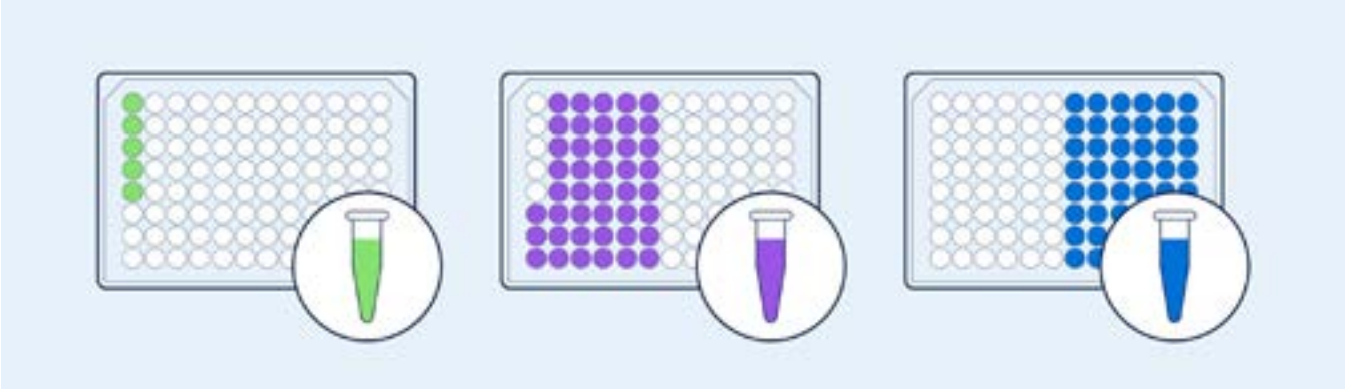


Figure 6. On-demand reagent flexibility in 96-well format. Scale at your own pace by running partial plates, using only the reagents you need, when you need them.

Scalable sample prep for FFPE tissue sections

Archival FFPE samples hold decades of research potential. High-throughput sample prep for single cell profiling makes it possible to unlock that value at scale, without the bottlenecks that have historically limited large studies. Our scalable Xylene-free protocol for FFPE sample deparaffinization and dissociation provides a faster, streamlined workflow option for sample preparation while maintaining high data quality across protocol formats and sample types (**Figures 7–9**). Individual tubes can be used for up to 16 samples, while the plate-based version can be used to process up to 96 samples in less than an hour.

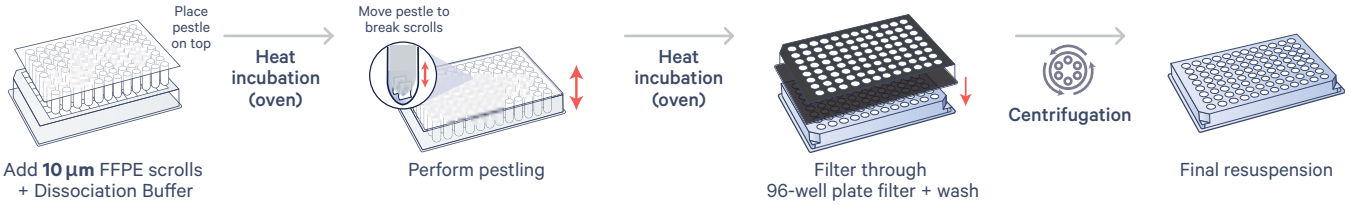


Figure 7. Scalable, Xylene-free, plate-based workflow for nuclei isolation from FFPE tissue sections. This Xylene-free protocol for FFPE tissue scrolls uses high-temperature incubation for deparaffinization and may be performed in tubes or in 96-well plates, enabling higher sample throughput in less time. FFPE tissue blocks ranging 1–20 years in age were used for demonstrating this protocol.

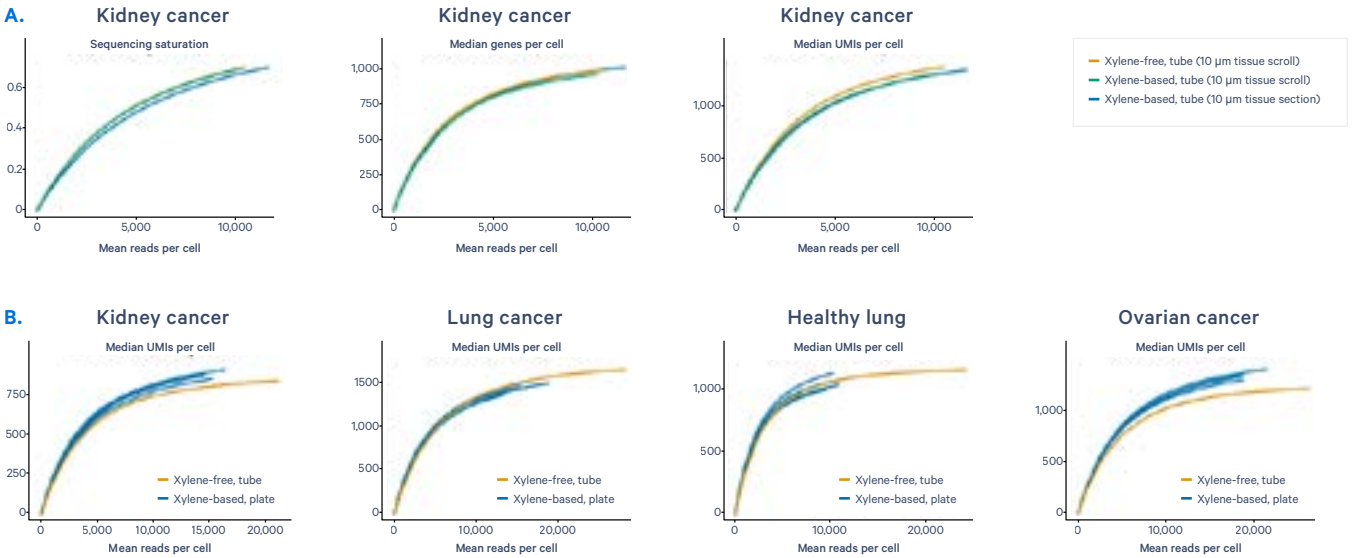


Figure 8. Xylene-free protocol maintains data quality for FFPE tissue sample analysis. A. The Xylene-free protocol for individual tubes maintains high data quality when compared with the Xylene-based workflow. **B.** Plate-based dissociation with the Xylene-free protocol demonstrates data quality parity with the tube workflow, across multiple tissue types.

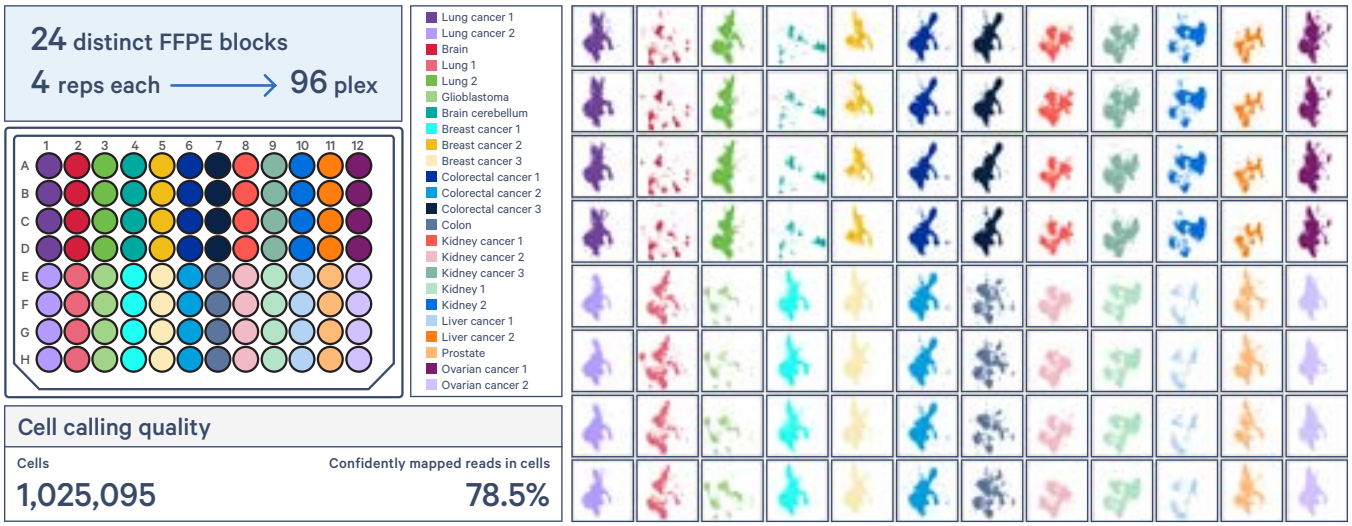


Figure 9. Apex enables high-throughput single nuclei RNA-seq (snRNA-seq) for FFPE samples with consistent data quality. The plate-based Xylene-free protocol was used to isolate nuclei from 24 distinct FFPE blocks, with four replicates each. Nuclei suspensions were then profiled according to the Apex protocol. Resulting snRNA-seq data showed consistent data quality across FFPE blocks and replicates.

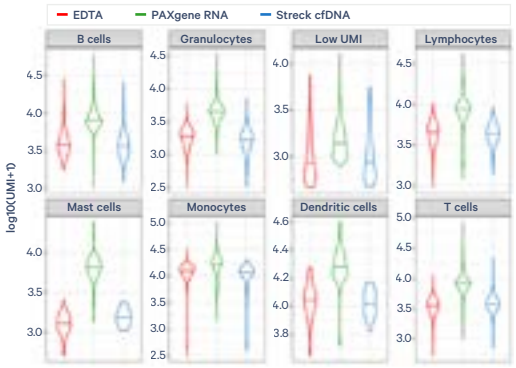
Convenient blood sample collection for real-world research

Single cell data from clinically derived samples can drive the discovery of novel biomarkers, investigations into disease progression, and more accurate views of drug mechanisms of action. But traditional sample collection workflows have not met the needs of multi-site or large-cohort studies. Immediate sample processing and access to a lab onsite put unnecessary hurdles in the way of transformational research.

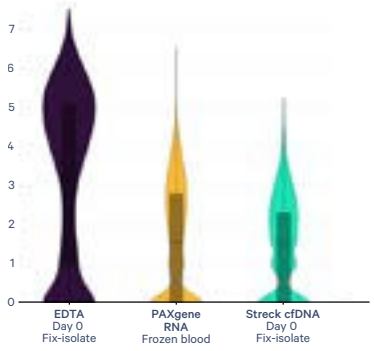
In contrast, novel protocols with Apex allow researchers to collect blood samples anywhere and process them when they're ready, while still maintaining high data quality (**Figures 10–12**). Blood samples are stabilized at the moment of collection, no need for lab access or intervention at the collection site. Depending on protocol selection, samples can be kept at room temperature or 4°C for up to 3 days, or frozen long-term before processing. With these Apex workflows, researchers can:

- Plan for future analysis with long-term storage between sample collection and processing
- Gain new insights with single cell analysis of frozen archival blood samples from previous studies
- Profile samples in a central location following distributed blood collection, without on-site processing
- Run longitudinal studies and time courses without introducing batch effects

A. Transcript abundance



B. Stress gene expression



C. Cell-type proportions

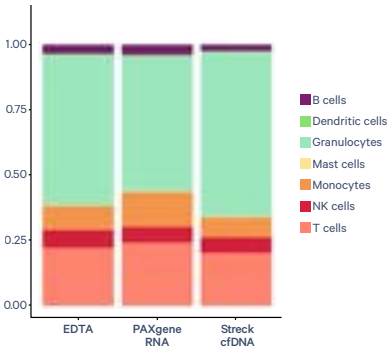


Figure 10. Apex blood analysis protocols demonstrate effective sample stabilization and maintain high data quality. A. Apex achieves higher UMIs per cell with PAXgene Blood RNA tubes (PAXgene RNA) compared to EDTA and Streck Cell-Free DNA Blood Collection tubes (Streck cfDNA) across two donors and all major immune cell types from both fresh and frozen blood. Streck cfDNA and EDTA samples show comparable high sensitivity. **B.** Stress gene expression was substantially lower in leukocytes isolated from PAXgene and Streck cfDNA samples than with EDTA, confirming effective transcriptional stabilization at the point of blood collection. (Fix-isolate: blood fixation was performed first, followed by cell-type isolation; "Day" indicates the number of days before processing.) **C.** Immune cell type proportions were comparable across EDTA, PAXgene RNA, and Streck cfDNA blood.

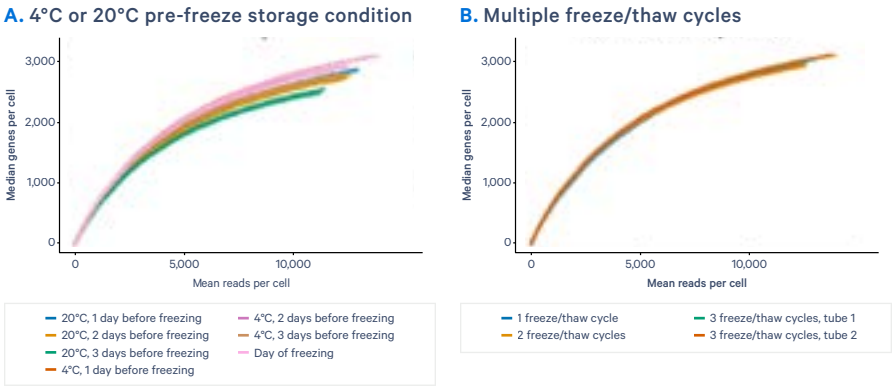


Figure 11. Apex blood analysis with PAXgene Blood RNA tubes enables sample storage and shipment, plus compatibility with multiple freeze/thaw cycles. A. Transcriptomic sensitivity was maintained for leukocytes across pre-freeze storage at 4°C or 20°C for up to 3 days. **B.** Data quality was also maintained with up to 3 freeze-thaw cycles (when storing at 20°C; testing on more freeze/thaw cycles is ongoing), eliminating the need to create aliquot tubes for follow-up studies and supporting PAXgene's logistical flexibility for multi-site workflows.

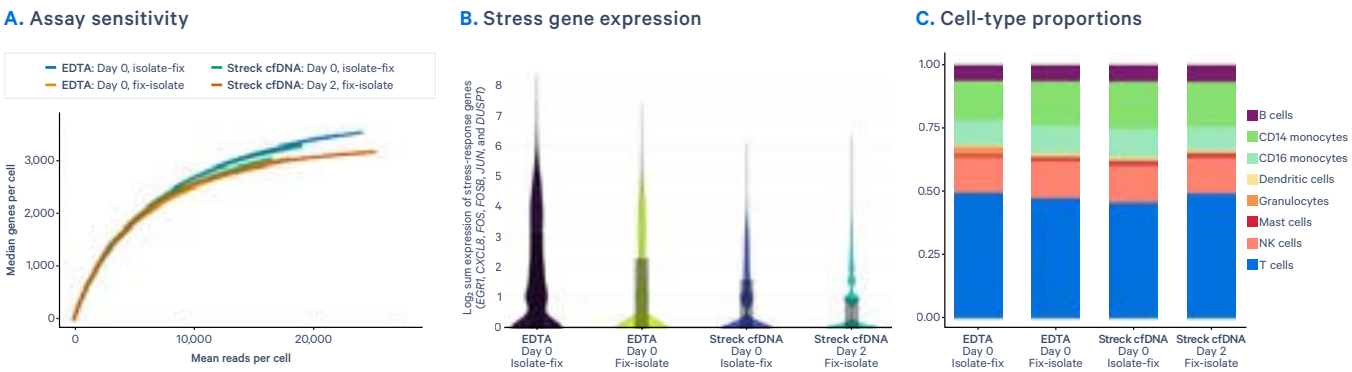


Figure 12. Apex blood analysis with Streck Cell-Free DNA Blood Collection tubes (Streck cfDNA) maintains data quality and sample biology. A. Transcriptomic sensitivity was maintained across PBMC samples with EDTA and Streck cfDNA methods. (Fix-isolate: blood fixation first, followed by cell-type isolation; isolate-fix: cell-type isolation first, followed by fixation; "Day" indicates number of days before processing.) **B.** Log_2 sum expression of stress-response genes (*EGR1, CXCL8, FOS, FOSB, JUN,* and *DUSP1*) was markedly lower in Streck cfDNA samples at Day 0 and Day 2 compared to EDTA samples, confirming effective transcriptional stabilization at collection. **C.** Immune cell-type proportions were largely comparable across EDTA and Streck cfDNA methods, indicating neither storage nor the blood fixation workflow distort cellular composition.

Cell surface and intracellular protein capture with Apex and Feature Barcode technology

Transcriptomic analysis provides a view on current cell types and potential states, while protein readouts can complement these data and provide insights into cell subpopulations and current functional states, as well as aid in mapping of signaling pathways. Combining transcriptomic and proteomic datasets from individual cells not only allows researchers to refine definitions of cell types and functional states for individual cells, but it also improves the resolution of transcriptionally similar cell subtypes, increasing the chances of discovering novel cell subtypes and rare cell subpopulations. Additionally, the ability to capture intracellular protein (a feature unique to our Flex assays) enables deeper investigations into cell signaling pathways that drive development, disease progression, and therapeutic response.

Apex supports single cell multiomic phenotyping through the simultaneous profiling of hundreds of cell surface proteins and/or intracellular proteins with Feature Barcode technology alongside gene expression analysis. The multiomic protocol follows the same general workflow as the standalone assay (**Figure 1**), with a few key modifications to sample preparation (**Figure 13)**, the micro-reactions that occur within the GEMs (**Figure 14**), and the dual index libraries (**Figures 15, 16**). Cells within the single cell suspension are labeled with oligo-conjugated antibodies. The antibody-conjugated DNA barcodes are then amplified within the GEMs for protein detection.

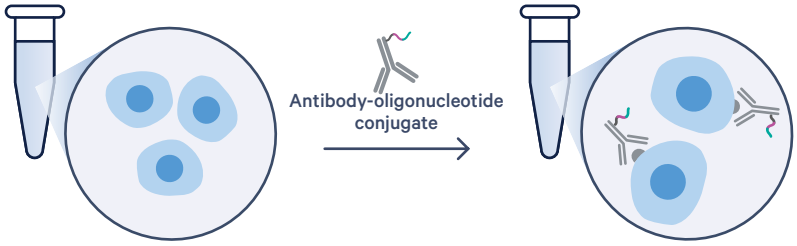
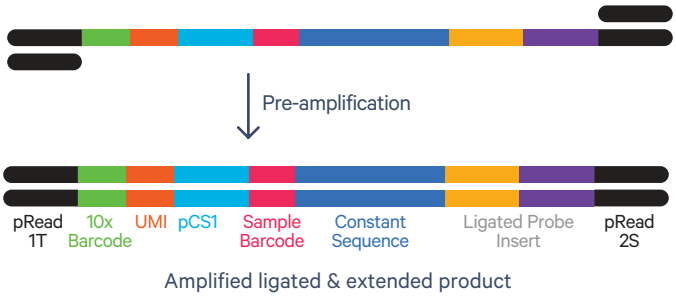


Figure 13. Sample preparation with Feature Barcode technology. Antibody-oligonucleotide conjugates can be added during sample preparation to label other molecules of interest, enabling multiomic readouts for the same individual cells. Note that this figure is specific to capture of cell surface protein alongside gene expression. Additional multiomic options for Apex include intracellular protein and CRISPR guide RNAs.

DNA pre-amplification: Gene expression



DNA pre-amplification: Feature Barcode

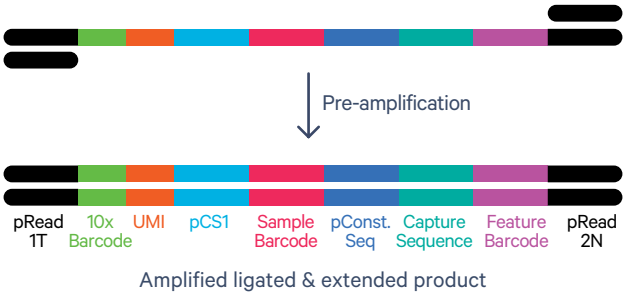
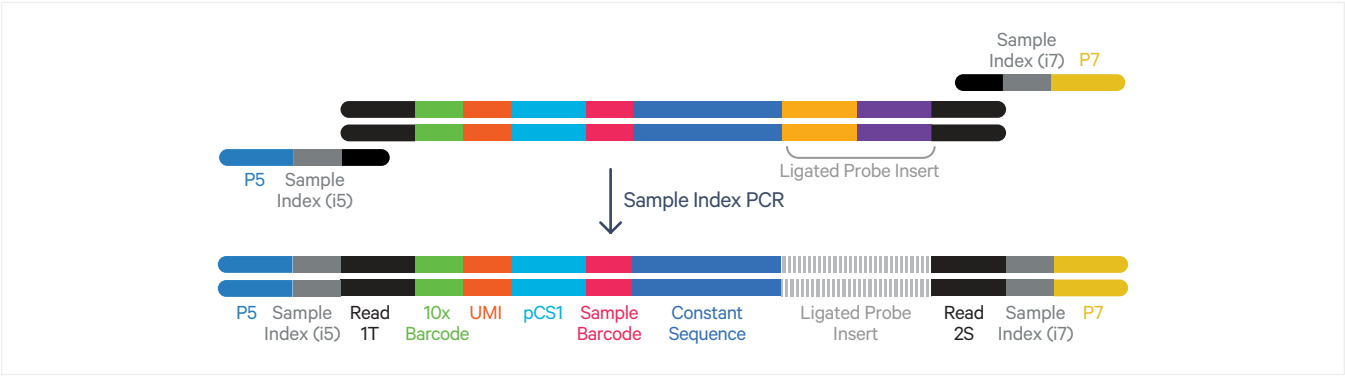


Figure 14. DNA pre-amplification. After GEM generation, the partitioned cells, Gel Beads, and Master Mix undergo ligation and barcoding steps. GEMs are then dissolved and pooled fractions are recovered. A PCR master mix is added for pre-amplification of the ligated and extended products, along with barcoded Feature Barcodes identifying proteins. Magnetic beads are used to clean up the pre-amplified products from the post-GEM reaction mixture, which includes leftover biochemical reagents and primers.

Pooled, amplified DNA processed in bulk: Gene expression



Pooled, amplified DNA processed in bulk: Protein expression

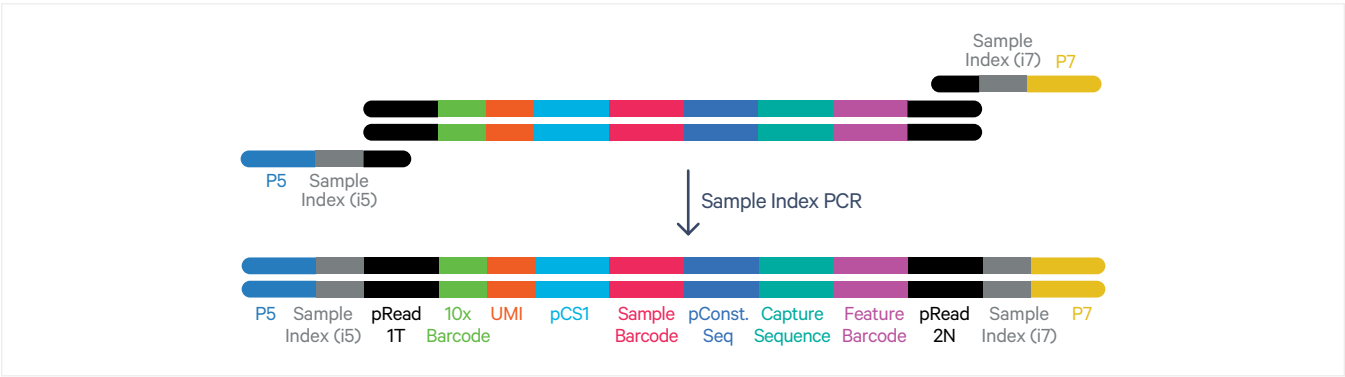
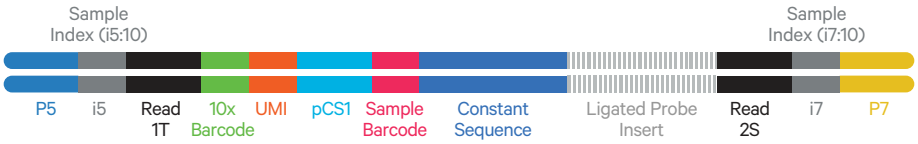


Figure 15. Pooled DNA amplification. The cell-barcoded DNA molecules from the pre-amplification steps are amplified via PCR to generate sufficient mass for library construction. Magnetic beads are used to clean up the amplified DNA molecules for construction of gene expression and protein expression libraries.

A. Apex gene expression library



B. Apex protein expression library

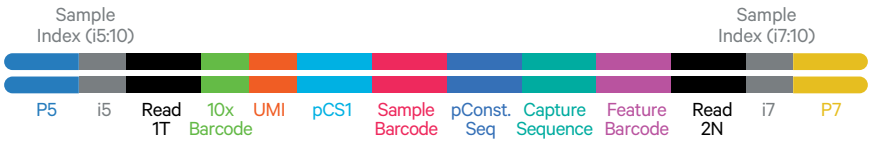


Figure 16. Apex dual index libraries. A. An Apex gene expression dual index library comprises standard Illumina paired-end constructs that begin and end with a P5 and P7 sequence. The 16-nt 10x Barcode and 12-nt UMI are encoded in Read 1T, while Small Read 2 (Read 2S) is used to sequence the ligated probe fragment. **B.** An Apex protein expression dual index library comprises standard Illumina paired-end constructs that begin and end with P5 and P7. The 16-nt 10x Barcode and 12-nucleotide UMI are encoded in Read 1T, while Nextera Read 2 (Read 2N) is used to sequence the antibody-conjugated DNA barcode.

Genome-wide CRISPR screens with VIPerturb-seq

CRISPR screening is a powerful approach to interrogate mechanisms underlying development, disease, and therapeutic responses. Single cell CRISPR screens extend this approach by providing the opportunity to directly assess CRISPR-driven gene edits or knockdowns and resulting gene expression phenotypes, cell by cell. Concurrent detection of single guide RNAs (sgRNAs) and perturbed gene expression profiles allows researchers to evaluate gene function across different cellular processes, assess genetic contributions to disease, or validate new drug targets.

Apex CRISPR with VIPerturb-seq (**3**) extends single cell functional genomics to genome-wide scales. A small, modular pool of barcode probes (fewer than 2,200 pre-designed sequences) enables detection of 1.1 million unique perturbations (**Figure 17**), providing a kitted solution in place of custom probe design and costly orders for a full genome-wide probe set from a third-party vendor. Apex CRISPR probes are incorporated directly into the probe hybridization step of the Apex workflow (**Figure 1**), allowing CRISPR sgRNAs to be captured alongside transcriptomic profiling in the same assay. To provide you with a more comprehensive view of each sample, genome-wide screening remains fully compatible with cell surface and intracellular protein detection. Plus, the automation-friendly workflow supports higher-throughput study designs. Together, these capabilities make genome-wide single cell screening practical for investigating disease modeling and drug interactions at scale.

The Apex workflow also supports a direct capture method with the use of custom, guide-specific probes. As with genome-wide screens, custom probe spike-ins are incorporated into the probe hybridization step (**Figure 1**), enabling downstream generation of single cell CRISPR libraries.

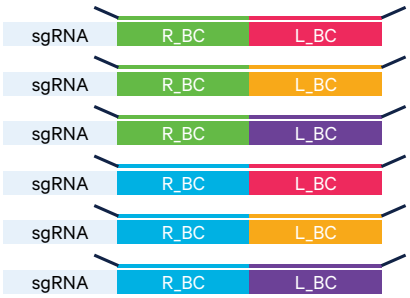


Figure 17. A small pool of spike-in probes makes genome-wide screens accessible and affordable. Modular barcoding with fewer than 2,200 pre-designed probes enables detection of up to 1.1 million unique perturbations alongside capture of gene and protein expression profiles.

Data analysis

10x Genomics software tools provide researchers with varying levels of bioinformatics experience access to an intuitive analysis pipeline. Cell Ranger and Loupe Browser software (available on the 10x Genomics Support website) enable users to analyze the sequencing data from Apex analyses.

Data analysis starts with Cell Ranger software, which allows researchers to:

- Process raw sequencing data to align reads and perform quality control analyses
- Identify cell barcodes to quantify gene and protein expression levels in individual cells
- Demultiplex samples from multiplexing workflows for Apex
- Generate cell feature matrices to analyze data produced using multiomic assays, such as Apex with Feature Barcode technology
- Perform clustering and secondary analyses, such as dimensionality reduction, cell clustering, and differential gene expression
- Automatically identify and annotate cell types based on standardized datasets with our Automated Cell Annotation* tool

Cell Ranger software has set the standard for single cell analysis software, with 9,500+ citations to date. Additionally, these pipelines are easily accessed via the 10x Genomics Cloud Analysis platform, a simple web interface that processes your data in as little as 90 minutes.

The desktop visualization tool, Loupe Browser, enables interactive data exploration of cell clusters and differential gene expression to aid data interpretation. With Loupe Browser, you can:

- **Find significant genes:** Determine genes that uniquely characterize clusters at the press of a button
- **Refine cell types:** Use gene lists and the gene expression view to refine cell types from Cell Ranger Automated Cell Annotation
- **Assess data quality:** Compare clustering resolution and performance across samples

Researchers can integrate data produced using our Next GEM and GEM-X Flex assays with chemistry batch correction, preventing interruptions to ongoing projects that include personal or publicly available datasets produced with Next GEM technology.

If further analysis is needed, researchers can seamlessly move between Loupe Browser and community-developed tools written in R using the conversion package, LoupeR. LoupeR also eases work with external collaborators or service providers that offer researchers in-depth bioinformatics expertise, including 10x Genomics Service Providers (SPs).

***Annotation is performed using a model that was co-developed by 10x Genomics and the Cellarium AI Lab at the Data Sciences Platform of the Broad Institute.**

Validation studies

The Apex assay delivers consistent performance across workflow options, even with increasing scale. To evaluate performance for large-scale studies, 384 human PBMC samples were processed simultaneously with our plate-based multiplex workflow. Samples were fixed and hybridized with the whole transcriptome panel and 384 unique multiplexing barcodes. Barcoded samples were pooled and loaded on the GEM-X chip, targeting recovery of up to 1 million cells per lane. Transcript sensitivity, as measured by median UMI counts, demonstrated consistent performance across 384 multiplexing barcodes, and cell clustering revealed expected cell-type populations (**Figure 18**).

For a direct comparison between our tube- and plate-based workflows, dissociated cells from fixed human testes were hybridized and processed according to the Apex protocol for either a 1.5 mL tube or a 96-well plate. Both workflows demonstrated exceptional performance, delivering comparable gene and transcript sensitivity, regardless of format (**Figure 19**).

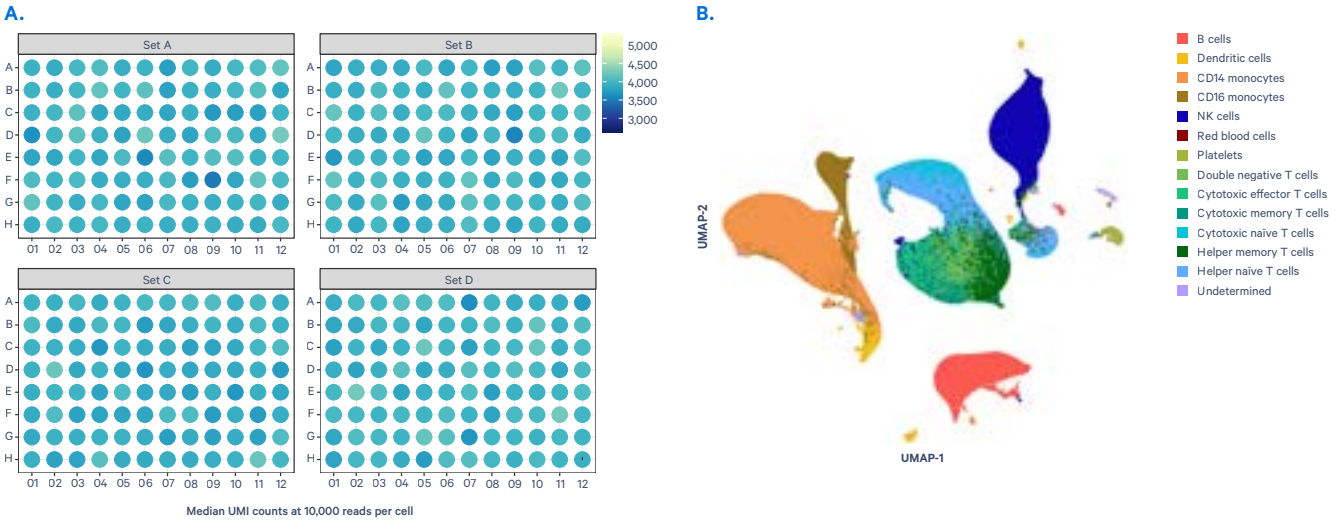


Figure 18. Apex enables simultaneous processing of up to 384 multiplexed samples and profiling of up to 1 million cells per lane with high consistency. Human PBMCs were fixed and hybridized with the human whole transcriptome probe panel and 384 multiplexing barcodes. Samples were then pooled and washed according to the Apex workflow. Approximately 1.45 million cells were loaded in a single lane, targeting recovery of 1 million cells. **A.** Consistent sensitivity (as measured by transcript abundance) is shown across 384 multiplexing barcodes. **B.** Gene expression UMAP shows cell-type clustering for 969,054 PBMCs recovered from this single lane.

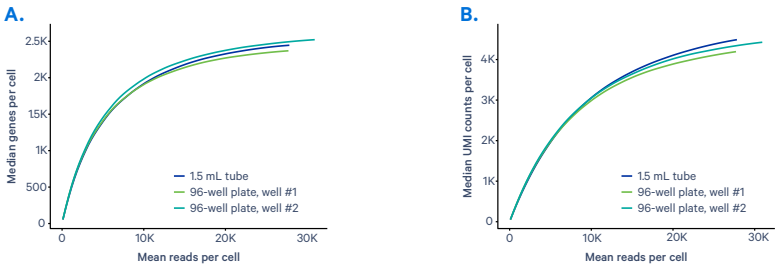


Figure 19. Apex provides consistent data quality and library complexity in tube and plate format. Cells dissociated from fixed human testes were hybridized and processed with Apex in either a 1.5 mL tube or a 96-well plate. 5,800 cells were loaded for each sample, targeting 4,000 cells each. Samples processed in tube and plate formats show comparable per-gene sensitivity (**A**) and transcript abundance (**B**).

In addition to high-quality transcriptomic data, Apex is ideal for deeper investigations into cell subpopulations and cell signaling pathways. Capture of cell surface protein sharpens cell-type definitions, resolves similar subtypes, and helps uncover rare populations. Intracellular protein allows researchers to go further still, providing insights into signaling pathways that drive disease and development. Our GEM-X Flex v1 assay previously demonstrated this utility, with a closer look at signaling pathways in kidney cancer. To evaluate protein capture with Apex (GEM-X Flex v2), human PBMCs were stained with two different antibody cocktails (one for cell surface protein, and one for both cell surface and intracellular protein) prior to fixation and profiling with the Apex assay. Gene and protein expression libraries were generated for each, and the resulting data show cell clustering informed by multiomic characterization (**Figure 20**).

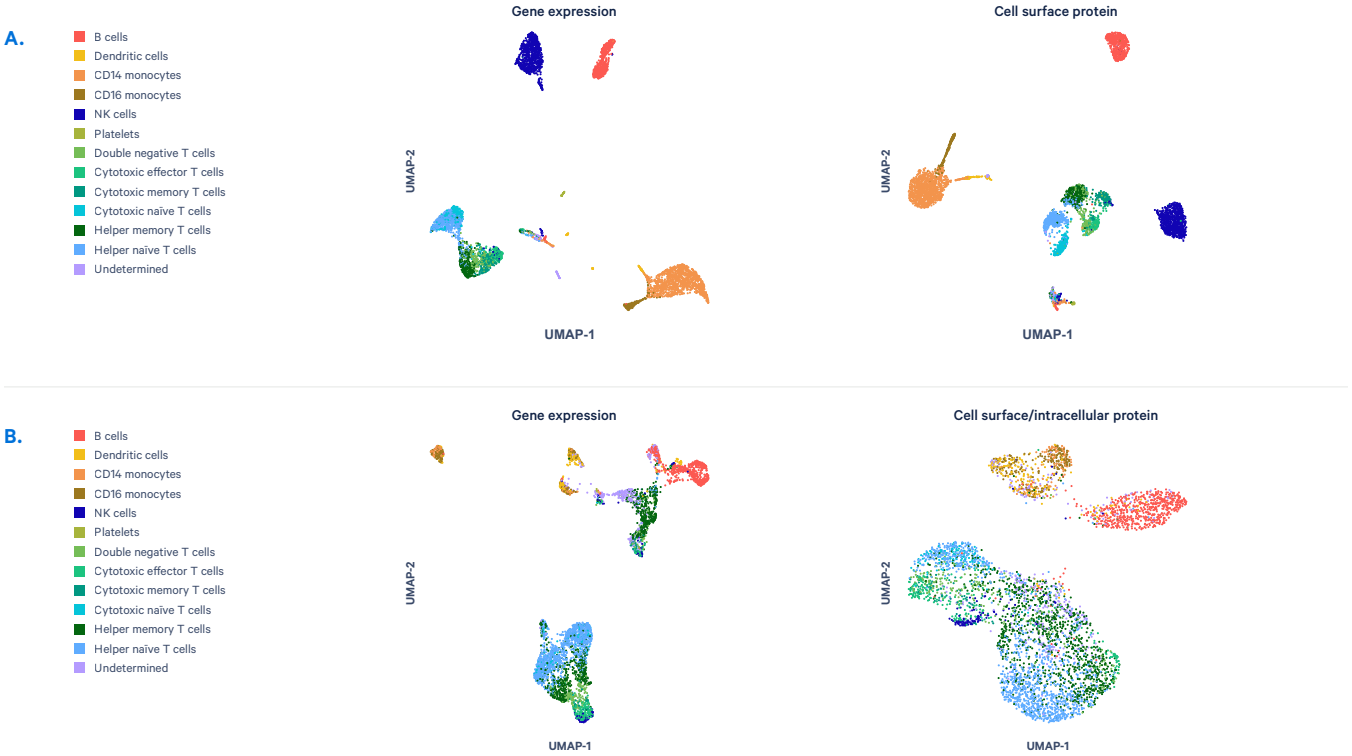


Figure 20. Multiomic characterization of samples with Apex for ultra-high parameter multiomic cytometry. Gene and protein expression UMAPs show cell clustering for human PBMC cell types after profiling with two different antibody cocktails. Stained cells were washed twice prior to fixation overnight at 4°C. For each stained and fixed sample, 25,000 cells were hybridized and processed with the Apex workflow (targeting 4,000 cells each). **A.** Human PBMCs were stained with 175 antibodies against cell surface proteins (TotalSeq™-C Human Universal Cocktail V2.0, BioLegend). **B.** An equal mix of rested (unstimulated), PMA/Ionomycin (PMA/Iono)–stimulated, and lipopolysaccharide (LPS)-stimulated human PBMCs were stained with 53 antibodies against cell surface and intracellular proteins (MultiPro™ Human Fixed Cell Immune Profiling Antibody cocktail, Proteintech Genomics).

Applications

From basic research to translational applications, Flex single cell assays have empowered researchers to ask bigger questions and find more meaningful answers. Key applications include biomarker discovery, therapeutic target identification, disease mechanism characterization, and deep profiling of clinically relevant sample types at unprecedented scale.

Browse our publication library to explore use cases from your peers >

Justification for using Apex for your research

Apex is ideally suited for single cell multiomic studies at every scale, with throughput capacity and workflow flexibility built to accommodate the demands of real-world research, including multi-site studies and large cohorts.

- **Streamlined and robust workflow:** Apex enables gene expression or multiomic profiling at single cell resolution using our tried-and-tested Chromium workflow.
- **Demonstrated technology**: Chromium assays have been used as a cornerstone technology in many peer-reviewed papers in high-caliber journals, including *Science*, *Cell*, *Nature Neuroscience*, *Nature Communications*, *Nature Protocols*, and others.
- **High consistency and sensitivity**: Validation studies demonstrate high consistency and data quality at scale, across tube- and plate-based workflows.
- **Protein capture:** Combined with Feature Barcode technology, Apex enables comprehensive profiling with simultaneous detection of cell surface and intracellular proteins alongside gene expression, enabling more refined cell-type definitions, detection of rare populations, and deeper investigations into signaling pathways.
- **Genome-wide CRISPR screens:** Pre-designed, modular barcode probes incorporated in the Apex workflow enable concurrent detection of single guide RNAs and perturbed gene expression profiles—fewer than 2,200 pre-designed probes support genome-wide screens of up to 1.1 million perturbations.
- **Optimized for diverse samples:** Apex delivers high performance across a wide range of sample types, as demonstrated with cell or nuclei suspensions, fresh or frozen tissue, FFPE tissue, PFA-fixed tissue, flow-sorted cells, fixed whole blood, and organoids.
- **Eased workflow logistics:** Flexible sample prep options accommodate the realities of diverse collection settings without compromising data quality. Samples can be fixed at the point of collection, stored, and batched for centralized processing, removing urgency from downstream workflows and making multi-site and longitudinal studies significantly easier to manage.
- **Large-scale studies:** Optional plate-based multiplexing enables profiling of up to 384 samples or 100 million cells per week. The 96-well format minimizes handling, supports automation, and improves consistency across experiments, making it well-suited for large studies, including high-throughput CRISPR screens.
- **Comprehensive data analysis solution:** Apex provides an easy-to-use data analysis pipeline as well as state-of-the-art software for data visualization. This empowers researchers with varied levels of bioinformatics experience to perform streamlined interpretation of transcriptomic profiles, cell clustering, and differential expression analysis for genes, proteins, and CRISPR perturbations.
- **Broad support resources:** 10x Genomics provides comprehensive support resources, ranging from technical specialists trained in Chromium single cell workflows to freely available videos and documents that guide users through the entire workflow.
- **Service Providers**: Get streamlined access to the complete Apex workflow through Service Providers, third-party facilities specially trained and verified by 10x Genomics to support a wide variety of single cell biology research applications.
- **Certified product quality**: 10x Genomics product development and manufacturing processes are ISO 9001:2015 certified.

References

1. Xu Y, et al. A single-cell transcriptome atlas profiles early organogenesis in human embryos. *Nat Cell Biol* 25: 604–615 (2023). doi: 10.1038/s41556-023-01108-w
2. Pilcher W, et al. Cross center single-cell RNA sequencing study of the immune microenvironment in rapid progressing multiple myeloma. *NPJ Genom Med* 8: 3 (2023). doi: 10.1038/s41525-022-00340-x
3. Bradu A, et al. Genome-wide single-cell perturbation screens with VIPerturb-seq. *bioRxiv* (2026) doi: 10.64898/2026.02.12.705613

Resources

- Single Cell Buyer's Guide
- Chromium Technology Brochure
- Flex Apex Product Sheet
- Software Tutorials
- 10x Genomics Cloud Analysis (check for availability in your region)
- Flex Apex Documentation
- Chromium X Series Instrument Brochure
- Publication Library

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