

10x Single Cell Gene Expression (3' or 5')

Product Overview

The 10x Genomics Gene Expression (GEX) assay enables transcriptional profiling at the single cell level across thousands of cells, providing insight into how cellular heterogeneity contributes to a biological system. Broad Clinical Labs (BCL) offers full library preparation and sequencing for GEX samples. Based on their needs, customers can choose to target regions closer to either the 3' end or 5' end of transcripts. Both assay versions can be supplemented with feature barcoding for selective targeting of cell surface proteins. The 5' assay can also be paired with a T or B cell enrichment to further investigate immune cells.

The GEX process utilizes gel beads fixed with specific barcodes, UMIs, and capture sequences designed to attach to either the 3' or 5' end of a full length cDNA molecule. Pools of these beads along with cell suspensions are processed through a microfluidic chip, with reverse transcription reagents, to create an emulsion of nanoliter-sized droplets called Gel beads in EMulsion (GEMs). Each cell in a GEM will bind to the bead then undergo reverse transcription, which barcodes each individual cell. Broad Clinical Labs has built a fully automated cDNA generation and library construction workflow for higher throughput, faster processing, and a level of data consistency and quality difficult to achieve in lower-throughput laboratories using manual sample preparation methods.

To minimize cell or nuclei damage, samples must be processed as soon as possible upon arrival at BCL's single cell lab. Customers should coordinate with their BCL Project Manager on sample arrival times to allow lab staff to schedule reagent preparation for 10x chip loading.

The BCL single cell team operates in the flow cytometry lab space at Broad's 75 Ames Street facility, which is more centrally located to public transport and main roads, as well as closer to users on the Broad campus. Due to the time sensitive nature of processing cells and nuclei, samples must be hand delivered rather than shipped.

All 10x samples are sequenced using BCL's "Walk-up Sequencing" workflow to enable customization of the depth of sequencing targeted for each sample, based on customers' unique cell types and quantities.

What's Included

- GEM creation from cell or nuclei suspension
- cDNA generation and library construction with available QC
- Sequencing and data delivery
- Data delivery to customer-owned cloud location in Google Cloud or Amazon Web Services; or Terra Workspace

Input Requirements

- Dissociated and filtered cells or nuclei, derived from fresh frozen tissue or PBMCs, suspended in 5-40µl of compatible buffers
- Optimal and strongly recommended: 10,000-15000 cells/nuclei in 15µL of 1X PBS (calcium and magnesium-free) containing 0.04% BSA.
- Hand delivery of samples to 75 Ames St. Cambridge, MA, with coordination of sample delivery times with BCL Project Manager

Data Deliverable

- Raw BCL files