



On-Chip Multiplexing addendum for 10X Genomics 3'GE scRNA-seq v4

This protocol is an addendum to the GECF Guidelines for 10X Genomics 3'GE scRNA-seq v4. It only includes differences from the 3'GE classical protocol (non OCM)

The GEM-X Universal 3' Gene Expression v4 4-plex assay provides a scalable microfluidic platform for on-chip multiplexing (OCM) of up to 8 samples (two sets of up to 4 samples each) to assess single cell 3' gene expression.

Differences from the standard 3' GE scRNAseq:

- **Cheaper** price per sample
- **Maximum 5'000 targeted cells/nuclei** (while standard protocol can target maximum 20'000 cells/nuclei).
- Optimal concentration is **1'500-2'000 cells/ul** (so higher than for regular 3'GE v4)
- **Minimal volume is 30ul** (so lower than for regular v4). As for v4, let us know in advance if you think you cannot reach this volume.
- **Multiplet rate** is 4x higher than standard method. We thus recommend not targeting more cells than needed.

Multiplet Rate (%) per Sample*	# of Cells Recovered per sample
~1.5%	~1,000
~3.1%	~2,000
~4.6%	~3,000
~6.1%	~4,000
~7.6%	~5,000

- It is a **4-plex assay**, explaining the pricing structure in “samples packages”.
- All 4 samples loaded in a single multiplexing set should have **similar RNA content** and **cell/nuclei number**.
 - o If the RNA content is expected to vary significantly between the samples in one multiplexing set, OCM is not recommended.
 - o Depending on experimental goals, if dissimilar samples are multiplexed, additional sequencing may be required to get sufficient saturation/data across all multiplexed samples.

Versions log

- vA.01 (14.03.2025): first release.
- vA.02 (19.03.2025): Modified the optimal concentration of cells to bring.
- vA.03 (25.03.2025): Mentioned minimal volume of 30ul.