



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 3' Gene Expression v4 On-Chip Multiplexing (OCM) assay allows to multiplex sets of 4 samples without requiring any labelling.

Technical differences from the standard 3' GE scRNA-seq:

- **Maximum 5'000 targeted cells** (while standard protocol can target maximum 20'000 cells).
- Optimal concentration is **around 1'500 cells/ul** (1'200-1'800 cells/ul), thus higher than in regular 3'GE v4.
- We can load **10ul max** in the chip for OCM (vs 40ul in regular v4), therefore the minimal volume to bring is 15-20ul instead of 50ul. **Therefore, if you expect the number of cells to be borderline, don't resuspend cells in more than 15-20ul. As for v4, let us know in advance if you think you cannot reach this volume and concentration.**
- **Multiplet rate** is 4x higher than standard method at a given targeted cell number. 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 **cells 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.
- vA.04 (14.10.2025): decreased minimal volume users should bring us (down from 30 to 20ul).
- vA.05 (20.10.2025): Clarified to resuspend cells only in 15-20ul if cell number is limiting.