protocol v1.05

CellPlex on scRNA-seq 3'GE v3.1

These guidelines are an addendum to our 10XG guidelines (available on our web site) and to 10XG cell/nuclei preps guidelines, which all should be read carefully.

Practical guidelines

- The labelling is done by users. We can provide tags if needed (at list price), but contact us/collect these reagents well in advance to avoid bad surprises.

- Refer to 10XG CG000391 user guide for labelling. If your cells significantly diverge from the ones mentioned in these guidelines, contact us or 10XG tech support for guidance.

- Collect necessary reagents in advance (in addition to the oligos tags).

- Careful washing after the labelling is critical to allow for efficient demultiplexing of the samples.

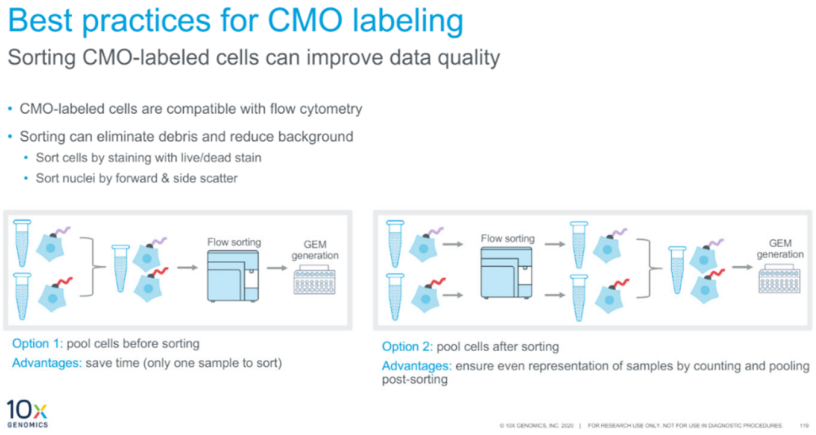
- The samples must be brought to GECF already pooled, and at the right concentration:

- For less than 10'000 targeted cells in total, the guidelines are the same as regular 3'GE 10XG: 700-1'200 cells/ul, but with an absolute minimum volume of 50ul (25ul not possible).
- For >10'000 targeted cells in total, the recommended cell concentration is higher: 1'300-1'600 cells/ul. Also with an absolute minimal volume of 50ul.

- it is expected that ca 50% of the cells will be lost in the labelling procedure, and it is not recommended to start from less than 100'000 cells (rather >150'000 if you target 30'000 cells). Especially fragile cells may suffer even more and CellPlex may not suitable in such case (a pilot labelling trial is recommended).

- Pooling ratio work best with similar amounts of cells for each sample, but down to 5% is supported by 10XG (below CellRanger may fail).

- CellPlex labelling is compatible with downstream FACS if needed:



- 10XG have not yet tested the CellPlex performances on frozen tissues.

- Mix conditions between pools (if several) to minimize batch effects.

- The CellPlex labelling procedure involves several washes/centrifugations and a 5 minutes RT° incubation, therefore this may impact on transcriptional profile of the cells. This should be kept in mind or checked by a control experiment if needed.

General description

- Based on lipids tagged with oligos

- Can be used on cells and nuclei

- Species-agnostic (plants not tested)

- Up to 12 multiplexed samples and 30k cells/channel

- Compatible with 3'GE scRNA-seq only (and surface proteins, CRISPR barcoding or targeted panels).

- Not compatible with 5'GE+VDJ (likely soon), or other methods (ATAC, multiome).

- 10XG haven't tried with methanol fixed cells, but they say "doubtful that it will work well".

- For more details regarding CellPlex technique and performances, a comprehensive tech note: https://support.10xgenomics.com/permalink/technical-note-chromium-next-gem-single-cell-3-v31-cell-multiplexing#header

Relationship between cells number, tags number and multiplets rate

- Multiplets rate increases with number of cells, and gets very high above 10k cells.

Targeted Cell Recovery	# of Cells Loaded	Cell Barcodes Detected	Singlets	Multiplets	Multiplet Rate
500	825	~500	~500	~3	~0.4%
1,000	1,650	~1,000	~1,000	~10	~0.8%
2,000	3,300	~2,000	~2,000	~40	~1.6%
3,000	4,950	~3,000	~2,900	~80	~2.4%
4,000	6,600	~3,900	~3,800	~140	~3.2%
5,000	8,250	~4,800	~4,600	~210	~4.0%
6,000	9,900	~5,700	~5,400	~300	~4.8%
7,000	11,550	~6,600	~6,200	~400	~5.6%
8,000	13,200	~7,500	~7,000	~510	~6.4%
9,000	14,850	~8,400	~7,700	~640	~7.2%
10,000	16,500	~9,200	~8,400	~780	~8.0%
12,000	19,800	~10,900	~9,800	~1,100	~9.6%
14,000	23,100	~12,500	~11,000	~1,500	~11.2%
16,000	26,400	~14,000	~12,100	~1,900	~12.8%
18,000	29,700	~15,500	~13,100	~2,300	~14.4%
20,000	33,000	~16,900	~14,100	~2,800	~16.0%
22,000	36,300	~18,300	~15,000	~3,300	~17.6%
24,000	39,600	~19,600	~15,800	~3,900	~19.2%
26,000	42,900	~20,900	~16,500	~4,400	~20.8%
28,000	46,200	~22,200	~17,100	~5,000	~22.4%
30,000	49,500	~23,400	~17,700	~5,600	~24.0%

At 30k targeted cells for instance, only 17'700 cells are in droplets containing a single cell.

- CellRanger can partially detect and eliminate multiplets owing to their multiple tags (not multiplets due to poor dissociation though). The ability of multiplets removal by CellRanger for a given total cell number increases with the number of tags included.

Number of Tags	Multiplets Detectable
2	50.0%
4	75.0%
8	87.5%
12	91.5%

→ therefore 10XG recommend this relationship between number of tags and total cells number:

Targeted Cell Recovery	Number of Tags
500-2,500	2
2,500-10,000	2-4
10,000-20,000	4-8
20,000-30,000	8-12

Table 2. Suggested number of tags for a given Targeted Cell Recovery

Practical example: For 30'000 cells targeted, do not use only 2 tags with 15'000 cells each, as you will get a very high number of multiplets (24%), of which only 50% will be identified and discarded by CellRanger, giving you at the end still 12% of unidentified multiplets in your data (way too high for proper analysis).

- The reads from multiplets are only eliminated by CellRanger but not rescued, therefore these reads must be considered accordingly when calculating the total number of reads needed and designing the sequencing runs (especially relevant if you plan to sequence deep).

- A practical example: For instance, if you target 20k cells, these 20k cells will be composed of 14'100 cells in droplets containing 1 cell, and the remaining 5'900 cells will be distributed in 2'800 droplets each containing several cells. Importantly, all these cells will be sequenced, and therefore all the 20k cells must be included in your total number of reads calculation. This amount of multiplets is very high, but:

  - with 2 tags, 50% of these multiplets are identified and bioinformatically discarded, and therefore 8% of multiplets remain in your data (still quite high).
  - with 8 tags, 87.5% of multiplets are identified and bioinformatically discarded, and therefore only 1.6% of multiplets remain in your data.

- In conclusion, these relationships are not trivial, and some thought has to be given to find the sweet point between number of tags and number of cells for your experiment.

Disclaimer

Regarding putative failure issues, CellPlex requires some clarification since the labelling procedure is done on user side and cannot be QCed by the GECF before processing cells. If the experiment fails, we can in principle (but not always) assess on which side was the problem:

- if GECF gets nice libraries for both “multiplexing oligos” and GEX, and normal/good quality sequencing, but if despite that there are issues assigning reads to samples, the issue is extremely likely to come from the labelling side. The most typical issue is suboptimal washes which lead to cross-contamination of tags between samples.
- if we get good GEX libraries but no/bad oligos libraries, the issue is very likely to be at labelling step. These oligos libraries are routine at GECF and are straightforward, therefore it is unlikely that we specifically failed these libraries and not the GEX ones. We wouldn’t be able to formally exclude an issue from our side without running a positive control library in parallel, but since it is not economically feasible to do so, we will have to assume the issue is on the user side, unless of course we spotted an issue on our side or have some doubt.
- if we fail to get both good oligos and good GEX libraries, the issue is rather on our/10XG side, unless cells were of very poor quality and we got green light from user to proceed anyway. With nuclei it’s more of a grey zone since their “viability” cannot be assessed.

Changelog:

- v1.01-v1.04: Added disclaimer regarding failures. Clarified calculations. Clarified that the labelling is done on user side. Clarified concentration - targeted number of cells relationship.

- v1.05: Mentioned to mix conditions between pools (if several) to minimize batch effects. Clarified further the relationship between multiplets rates, number of cells and number of tags.