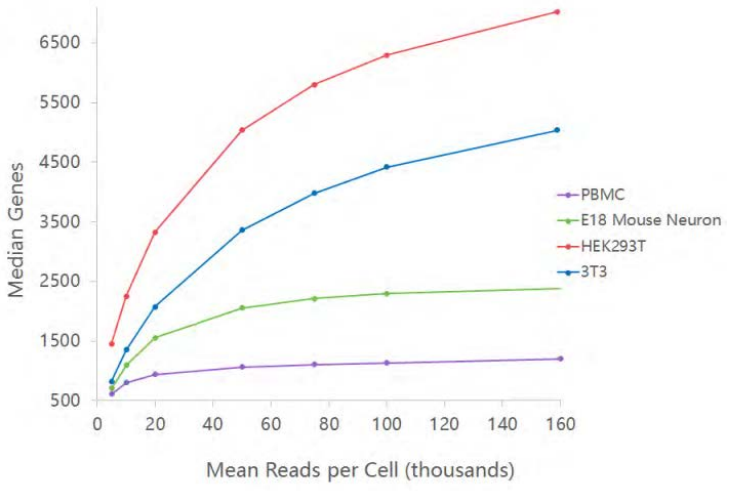
10x Genomics Chromium samples preparation guidelines

Important notes

Cell size

- Max cell diameter officially supported by 10x Genomics (10XG) is 30um, but the max theoretical limit is 65um (=size of the microcapillaries). These diameters are calculated for cells in suspension, not flattened on a petri/tissue surface.
- If your cells are at the upper limit of this size range, we can use “cheap” training beads to assess cells incorporation inside droplets with a microscope. In case your cells are too big, a special protocol exists for nuclei instead of cells. Nuclei isolation can also be useful when getting intact dissociated cells is not possible.
- Number of genes detected directly depends on the cell size (=amount of mRNA molecules per cell). Sequencing depth chosen may thus be modulated according to cell size. See plot from 10XG documentation:



Targeted cell number

- Define the number of cells you would like to be ultimately sequenced (=”targeted recovered cell number”), and indicate it to us when bringing the samples. Recovery rate is uncertain, in particular during first experiments with a given sample type, therefore target cells number is only indicative and number of actual recovered cells can differ significantly from it. See also comments in *Cells concentration* paragraph.
- The rate of doublets increases with targeted number of recovered cells (see table below), therefore we recommend not targeting more than 5’000 cells unless really necessary.

Nb of Recovered Cells	Multiplet Rate (%)
500	~0.4%
1 000	~0.8%
2 000	~1.6%
3 000	~2.3%

4 000	~3.1%
5 000	~3.9%
6 000	~4.6%
7 000	~5.4%
8 000	~6.1%
9 000	~6.9%
10 000	~7.6%

- Each additional recovered cell comes with a sequencing cost, therefore calculate the number of cells needed thoughtfully. Consider as well that once the sample has been processed, one cannot choose to sequence only a fraction of the recovered cells (technically impossible). The number of targeted cells depend on the biological question. Inquire if needed.

Diverse notes

- The standard protocol is intended for fresh unfixed/unfrozen cells, but a special protocol exists for methanol-fixed cells. Inquire if interested.
- If a mRNA from an ectopically-expressed transgene is highly overexpressed (GFP…) it may take a significant fraction of the reads, therefore take that into account when designing your experiment.
- Cells must not be infected with infectious agents, and primary human cells must have been screened for absence of such agents (HIV, HCV…). In addition, the sample should not contain toxic chemicals. Contact us in advance if it is not possible.
- If VDJ reconstruction is needed, tell us in advance as the whole method/kit is different.
- We can split the processing of cells in the instrument in several batches if this avoids some samples waiting on ice for very long times, and if this does not introduce batch effects.
- If you plan a big experiment, it may be worth first running 1 sample to assess quality of data and cell recovery rate.

Cells preparation

Also Refer to the last version of 10XG Cell Prep guide (document CG00053).

Viability

- The key word for the 10XG Chromium is cell viability. It is very important to get good results downstream.
- MACS-sorting in general leads to higher viability than FACS-sorting, so prefer the former if possible.
- If you sort the cells by FACS before loading, include a viability stain to get rid of dead cells if possible.
- Miltenyi sells a kit to remove dead cells if needed (“Dead Cell Removal kit”, 130-090-101), which works on mammalian cells and probably also on insects cells but to be tested to be sure.
- Before loading cells in the instrument, we'll check viability of the cells.
- The percentage of dead cells that can be considered acceptable depends on your experimental settings, and to which extent it can affect your downstream data analysis. Dead cells can generally be excluded

bioinformatically as they contain a high percentage of mitochondrial mRNAs, but it may not be done easily if cells have just died or are in the process of dying. If your cells come from a healthy suspension cell line, anything more than 5-10% dead cells is probably a sign that something is bad, while if working from primary cells that underwent hours of dissection and sorting, 20% dead cells may be considered acceptable. We will move forward with any sample containing less than 20% dead cells, unless the user explicitly decides otherwise. When samples contain more dead cells, it is the user decision to either move forward anyway, or perform a dead cells removal procedure (see above), or cancel the experiment and try to improve cell viability for a subsequent experiment.

Dissociation

- Cells should be well dissociated from each others, and if relevant carefully gated on FACS to avoid doublets.
- High EDTA is not OK here as it will impede reverse transcription, so if you use EDTA to detach cells you will have to quench it and do an additional wash.
- To avoid clumps, cells can be passed through a cell strainer. This is usually not necessary if cells are FACS-sorted. Some examples:
  - BD Falcon tube with 35um strainer, #352235, not ideal for small volumes. We have it.
  - Flowmi pipette cell strainer of 40um or 70um. We have the 40um ones.
  - Miltenyi Biotec 30 um PreSeparation Filter, cat#130041407….
- For dissociation of difficult tissues, consult publications from the relevant field and contact 10XG tech support. Using a GentleMACS instrument may help, as well as accutase, collagenase, pronase or even better psychrophilic proteases which work at 4° (Potter lab, PMID: 28851704).

Cell concentration

- Your cells concentration should ideally be around 700-1’200 cells /ul (incl dead cells). FACS sorters often overestimate cells concentration, therefore take some safety margin and ask the sorting facility to give you a concentration a bit higher than that. 25ul are the minimum, but the more the better since we will also have to do a trypan blue… so if possible bring >50ul or even more. If you think you cannot reach these values, please contact us, as there is room for some flexibility. Nevertheless, concentrations range outside these values will make target cell number even less predictable.
- For concentrating or washing cells, spin at 300xg for 5min at RT° for small cells (5-10um), 250xg for 4min for medium cells (10-17um), and 150 xg for 3 min at RT° for larger cells (17-25um). This will insure efficient pelleting while minimizing pelleting of debris. When doing it the first time, keep and check also supernatant to insure these settings are working for your cells.
- Before loading cells in the instrument, we'll double-check the cell concentration.
- Bring some extra medium to perform cells dilutions if needed.

Diverse

- Cells should be kept in their preferred medium (FCS is not a problem, as long as it was filtered to remove putative large particles).
- Perform one wash, as described in the 10XG cell prep guide. This wash can be performed with your culture

medium. In case cell amount is too low, this wash can exceptionally be skipped. More details in the cell prep guide.

- Place cells on ice once prepared.

Versions log

- v1.01: initial release.
- v1.02: increased the minimal volume of cells to be submitted to 25ul. Clarified that toxic chemicals or infectious agents must not be present in the samples. Mentioned Miltenyi kit for dead cells removal.
- v1.03: added plot with gene number depending on cell size. Clarified that the dead cells removal kit works on all mammalian cells.
- v1.04: Clarified that above 20% dead cells, it is the user decision to move forward, perform a dead cell removal, or cancel the experiment.
- v1.05: Clarified that “recovery rate is uncertain, in particular during first experiments with a given sample type, therefore target cells number is only indicative and number of actual recovered cells can differ significantly from it. See also comments in *Cells concentration* paragraph”. Also clarified that cells concentrations range outside recommended values will make target cell number even less predictable.