PROTOCOL: Cell fixation and preservation (methanol) for 10x Genomics single-cell transcriptomics

10/16/2020

FIXATION

1. Following cell harvesting, collect the cell suspension in a 15-ml tube, carefully count cells using a hemocytometer and centrifuge at 400g for 5 min at 4°C.
2. Aspirate the supernatant from the tube and discard, without disturbing the cell pellet.
3. Centrifuge the cells to be methanol fixed at 400g for 5 min at 4°C.
4. Aspirate and discard the supernatant. Resuspend the cell pellet in 1 ml of ice-cold 1X DPBS per $1x10^6$ cells (1ml minimum).
5. Centrifuge the cells at 400g for 5 min at 4°C.
6. Aspirate and discard the supernatant and resuspend the cell pellet in 200 µl of ice-cold 1X DPBS per $1x10^6$ cells (200ul minimum).
7. Add 800µl of methanol per $1x10^6$ cells (800ul minimum) (pre-chilled to −20 °C) dropwise while gently mixing the cell suspension by gently flicking the tube.
8. Place the cell suspension on ice for 30 min prior to transferring to −80 °C for long-term storage. Using this methanol fixation approach, high-quality single-cell transcriptomes from cells stored for several weeks to 12 months have been reported.

For 10x Genomics-based single-cell processing fix a minimum of 150,000 cells per sample. For longitudinal analyses, methanol fixation of cells at each time point, capturing cells and performing library preparation in one batch is recommended.

REHYDRATION

1. Following harvest of all samples in the study, rehydrate methanol-fixed cells by first placing them on ice for 15 min to equilibrate them to 4°C.
2. Centrifuge cells at 1000g for 5 min at 4°C and then carefully remove and discard the supernatant.
3. Resuspend cells in 0.2ml Wash-Resuspension Buffer (0.04% BSA + 1 mM DTT + 0.2 U/µl RNase inhibitor in 3X SSC).
4. If large cell clumps are visible pass cells through a 40-µm cell strainer prior to counting.
5. After counting centrifuge cells at 1000g for 5 min at 4°C, remove and discard the supernatant.
6. Based on the starting cell concentration and assuming approximately 50% cell loss, add an appropriate volume of Wash-Resuspension Buffer to obtain the desired cell concentration. This will mean gently pipette mixing using a regular-bore pipette tip to obtain a suspension of 2,500 cells per µl.
7. To minimize Wash-Resuspension Buffer carryover, it is recommended to use ≤4ul cell suspension stock (10,000 cells if at 2,500 cells per µl) when preparing the cell suspension for GEM Generation and Barcoding Step of the 10x Genomics Single Cell protocols.
8. Process cells for single-cell capture, library preparation and sequencing using 10x Genomics or Drop-Seq platforms, according to standard protocols.

Based on:

1. Alles *et al*. Cell fixation and preservation for droplet-based single-cell transcriptomics. *BMC Biology*, volume 15, Article number: 44 (2017).
2. Kong *et al*. CellTagging: combinatorial indexing to simultaneously map lineage and identity at single-cell resolution. *Nature Protocols*, volume 15, pages750–772 (2020).
3. 10x Genomics. DEMONSTRATED PROTOCOL (CG000136 • Rev E). Methanol Fixation of Cells for Single Cell RNA Sequencing.

   https://assets.ctfassets.net/an68im79xiti/7rsw40AVqX3ZXwIl7MDj85/fb7ac4e1b324827f5b738ade5a02b650/CG000136_Demonstrated_Protocol_MethanolFixationCells_RevE.pdf

The protocol was demonstrated with human synovial arthroplasties.

It is important to note that data quality from fixed samples may vary by sample type. Our protocol is expected to be compatible with many, but not all, cell or tissue types. Cell and tissue types that are particularly fragile and challenging to dissociate (e.g. organoids, biopsies, primary cancer cells, solid tissue) may require additional optimization to help to preserve the integrity of the transcriptome.