Single Cell Multiome ATAC + Gene Expression

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I. Steps in pre-processing: Thawing Frozen Cells

1. Prepare and label a 50 mL tube consists of 10 ml R10 media and warm at 37°C.
2. Warm the cells in a water bath (37°C). Keep a close eye on your vials; when there is still one ice crystal left on the external surface of the vial, remove the vial from the water bath.
3. Quickly transfer the cells to labeled 50 mL tubes.
4. Rinse the original vial to increase the number of cells you recover, rinse the cryo-vial with 1 mL warm medium and pour it into the 50 mL tube.
5. Centrifuge at 400g for 5 mins at 4°C. Discard the supernatant.
6. Add 1 mL ice-cold staining buffer and transfer cells to 5 ml FACS tubes. Keep cells on ice.
7. Optional: Proceed with sorting to remove dead cell/debris/granulocytes. It has improved data quality in our hands.
8. Count the cells if the cell number is high, adjust to dispense (1.5-5 million cells/mL) cells in 5 ml FACS tubes.
9. Centrifuge at 400g for 5 mins at 4°C. Discard the supernatant.
10. Aliquot 1 million stained cells into a 1.5 mL low bind tube and proceed to nuclei isolation protocol. Buffers for nuclei isolation should be prepared fresh on the day of experiment and keep the buffers on ice.
11. Continue with the multiome protocol.

II. Links to the protocols:

1. Nuclei Isolation for Single Cell Multiome ATAC + Gene Expression Sequencing
   https://www.10xgenomics.com/support/single-cell-multiome-atac-plus-gene-expression/documentation/steps/sample-prep/nuclei-isolation-for-single-cell-multiome-atac-plus-gene-expression-sequencing

2. Chromium Next GEM Single Cell Multiome ATAC + Gene Expression: https://www.10xgenomics.com/support/single-cell-multiome-atac-plus-gene-expression/documentation/steps/library-prep/chromium-next-gem-single-cell-multiome-atac-plus-gene-expression-reagent-kits-user-guide