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| Cell sorting before 10x Genomics **(in collaboration between SCS and Flow Cytometry Core)**  **Discuss the experimental set up not only with SCS but also with Flow Cytometry to make sure you have proper reagents (e.g. antibodies) and know the required flow time**  ***Goal: To enrich for specific cell types based on cell surface markers\****  **Alternative approach:** If using Miltenyi Biotech beads for cell fractionation, consider the differences compared to cell sorting:   * Purity is lower * Unable to select cells with particular expression level of the marker (high, intermediate, low) * Does not work with GFP-positive and similar cells * Viability if higher (the procedure is faster and no pressure applied)   *Notes: Cells tend to be more fragile after sorting. Handle cells gently to preserve cell heath and viability, which is critical for success of a 10x experiment. Cell loss during cell sorting is common. Start with higher cell number.*  **Procedure (based on 10x recommendations):**   1. Stain cells using appropriate protocol for cells/antibody, minimizing washing and centrifugation steps. For example, do not wash cell between the DNA dye and the antibody staining. 2. Sort based on purity as opposed to recovery, unless you need to increase cell viability. Aim to remove dead cells during sorting. Use a larger flow nozzle (e.g. 100µm) or run at 20 psi (or both). The flow rate may need to be increased if your cell population of interest is very low (<0.01%) or sorting a lot of cells, so that the sorted cells do not sit for an extended period of time before running the assay. 3. Sort into a low volume and directly into the solution that you will use for loading on 10x Genomics instrument (e.g. PBS/0.04% BSA). Keep cells on ice from this point.   *Notes:* 10-20 mln cells/ml give great data.  30-60 µl is the “dead” volume of the instrument.   1. Promptly load cells on 10x Genomics instrument.   *Don’t rely on cells counts from FACS as they are often overestimated (minimum 25%). Recount cells after sorting using hemocytometer.*  *Checkpoints:* Counting with Trypan Blue.  Immunofluorescent labeling. | **Reagents:**  Use Sneath fluid that has  < 0.1 mM EDTA  < 3 mM Mg2+.  consult the Flow Cytometry Core  Fluorescent antibodies of interest  (optimize dilution)  Always include  DNA dyes for dead cells, which are compatible with the antibodies used for sorting. See info about Zombie Dyes at  <https://www.biolegend.com/en-us/live-dead>  (optimize dilution)  PBS/0.04% BSA  10 mL:  9.99 mL 1x PBS  40 µL BSA 10% (Sigma, # A1595)  **Instruments:**  MoFlo (Astrios) cell sorter is designed for fast sorting. Still, sorting 10 mln cells will require ~90 minutes (consider cell viability)  *Book the sorter with Flow Cytometry Core 2 weeks in advance. An FCC staff person will be available to run your samples. Promptly collect the samples once the run is finished.* |

***\**** *If you have performed scRNA-seq already, you may be able to identify a cell surface marker for the population of interest. In this case, you can also use the FFC facility. Cell sorting based on this marker may be used for assays that don’t distinguish between individual cells (bulk) or for additional scRNA-seq focused on that cell population!*