Cell Preparation Guide for Single Cell Sequencing Projects

Sample Quality

- ☑ minimal cell debris
- ☑ no cell aggregates
- ☑ at least 70% cell viability
- ☒ *dying cells increase ambient RNA contamination & cell clumping*

Sample Handling

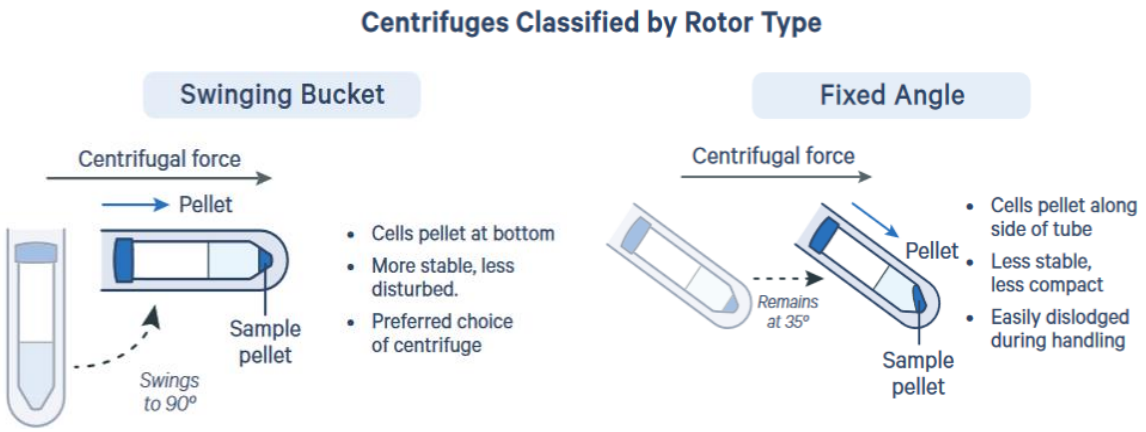
- ☑ place samples on ice after resuspension/sorting ❄
- ☑ store sorted/extracted samples not longer than **30 minutes** on ice before handing over the single cell core facility
- ☒ *prolonged sample handling negatively impacts sample quality*

Pipetting

- ☑ Speed: pipette cell suspensions slowly and gently
- ☒ *fast pipetting causes physical damage to cells by shearing forces*

Centrifugation conditions

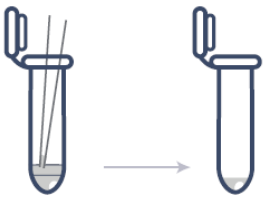
- ☑ adjust centrifugation conditions by to sample type
  - **nuclei:** 500 rcf, 5 - 10 min
  - **small cells:** 300 rcf, 5 min
  - **large cells:** 150 rcf, 3 min
- ☑ avoid excessive centrifugation
- ☑ swinging bucket centrifuge is preferred over fixed angle



10X Genomics

Washing and Resuspension

- ☑ optimize buffer conditions: 1X PBS (Ca and Mg free) + 0.04% w/v BSA (up to 1% w/v BSA) is recommended for most general sample preparation
  - ⚠ *for BEAM-Labeling: use 1X PBS + 2% FBS*
- ☑ optimize buffer volumes, number of washes and centrifuge conditions to reduce cell loss and debris
- ☑ always leave behind ~50 µl supernatant to preserve pellet after centrifugation



- ☒ *discarding the entire supernatant during washing and resuspension steps may cause pellet disruption and massive cell loss*
- ☒ *buffers should not contain >0.1 mM EDTA, >3 mM magnesium or surfactants as these interfere with reverse transcription and GEM generation*

Straining and Filtering

- ☑ microfluidic channels of 10X Chromium X are <100 µm wide: use pore size of strainer that is larger than the maximum cell diameter, but small enough to catch larger clumps
- ☑ filter at the last wash step and not the final cell suspension