UNIVERSITY OF LIVERPOOL | CENTRE FOR GENOMIC RESEARCH

Guidelines for preparation and submission of cells for Chromium 10x libraries

10x Genomics® Single Cell Protocols require a suspension of viable single cells as input. Minimizing the presence of cellular aggregates, dead cells, non-cellular nucleic acids and potential inhibitors of reverse transcription is critical to obtaining high quality data.

Cell preparation guidelines can be found here:
10x Genomics Cell preparation guide
10x Genomics Cell counting flowchart

Important considerations

- Keep the cells on ice at all times.
- Treat the cells gently throughout. This includes gentle pipetting (possibly by use of wide bore tips) and use of low centrifugation speeds.
- Keep the sample preparation time to a minimum. Some cells can be fragile and cell viability can significantly decrease if not processed immediately.
- Wash cells with a PBS + 0.04% Bovine Serum Albumin solution to remove contaminants, such as ambient RNA and unwanted buffer components.
- For more information on how variation between similar samples can influence the outcome of your experiment, please check the document, which can be downloaded from this link:
  10x Genomics Biological and technical variation in single cells

Important cell quality control measures

- Visually inspect the cells under a microscope to ensure that you have isolated a *single cell population*.
- Use a cell strainer to *ensure that cells are completely dissociated and free from debris and aggregates*. Clumps of cells and debris will block the channels of the 10x chip.
- Ensure that the cells are counted in triplicate due to the importance of an *accurate cell count to avoid under- or overloading the chip*. Remember to always *count the cells after sorting*.
  Please check the document, which can be downloaded from this link for more information:
  10x Genomics Guidelines on accurate target cell count
- Ideally, viability should be 90% or more, however, 10x Genomics recommend that it is certainly not lower than 70%. Dead cells contain a lot of contaminating ambient RNA which will interfere with the subsequent library preparation. If the viability of the cells is not sufficiently high, consider removing dead cells following the protocol, which can be downloaded from this link:
  10x Genomics Technical Note on Dead cell removal