Single Cell/Nuclei Isolation from Fresh Frozen Tissue

Scientific Relevance

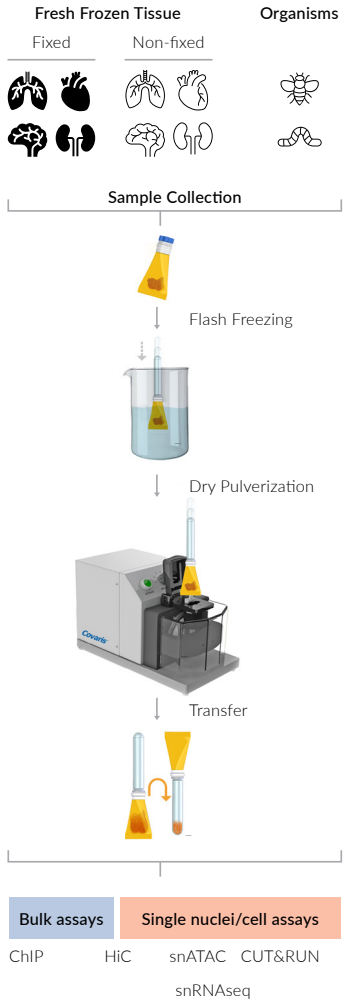
Bulk profiling of tissue samples mostly resemble transcriptome, proteome or epigenome of the most abundant cell type and averages across a heterogeneous cell population. However, deciphering cell to cell variability has huge potential to better understand biological and disease processes and is essential to develop better disease stratification and therapeutic intervention. Therefore, single cell/nuclei profiling methods have gained more attention and are constantly improving.

Challenges

Fresh samples in clinical research are difficult to achieve and the repository of fresh frozen samples is much bigger. However, many sample disintegration methods from fresh frozen tissue:

- produce heat during mechanical sample disruption interfering with preservation of biomolecules and/or organelles especially when starting from non-fixed samples
- depend on cumbersome, time consuming, manual mechanical disruption which is difficult to standardize between samples and operators
- do not maintain cell/nuclei integrity, especially from native starting material
- require direct contact with the sample which introduces the risk of sample contamination and loss

Workflow



Schematic representation of the CryoPREP workflow for nuclei/cell extraction by contact-free sample pulverization. Sample inputs such as fixed or non-fixed tissues or organisms are collected and placed in the centre of the tissue tube. Subsequently samples are flash frozen by submerging the bottom 2/3rd of the tissue tube in liquid nitrogen. Liquefied air is allowed to bubble out for 2 to 3 seconds followed by immediate insertion of samples into the cryoPREP instrument. Samples are pulverized via impacting with respective power and the cryofractured specimen is transferred directly into respective sample vessel for downstream processing.

Advantages of Covaris cryoPREP®

cryoPREP provides a fast, tuneable, efficient, reproducible, and contact-free sample pulverization.

- Contact-free and closed processing avoids potential cross contamination of samples
- Cryofracturization with adjustable controlled mechanical force ensures reproducible disruption of extracellular tissue matrix by minimizing sample loss ensuring ideal nuclei/cell isolation from diverse tissues
- Buffer-free, fast, controlled and cold processing enables unique preservation of cells, nuclei and biomolecules
- The fast and easy handling enables nuclei isolation in suitable batch sizes
- Besides cell/nuclei isolation the cryoPREP-based sample disintegration can be transferred to other organelle isolations such as liver microsomes for pre-clinical ADME/Tox studies

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Fixed Tissue

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