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Setting up single nucleus sequencing

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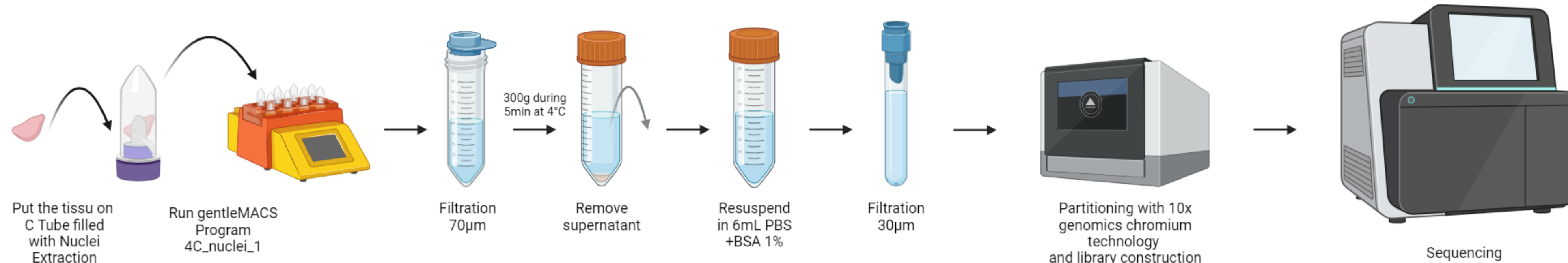
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Abstract

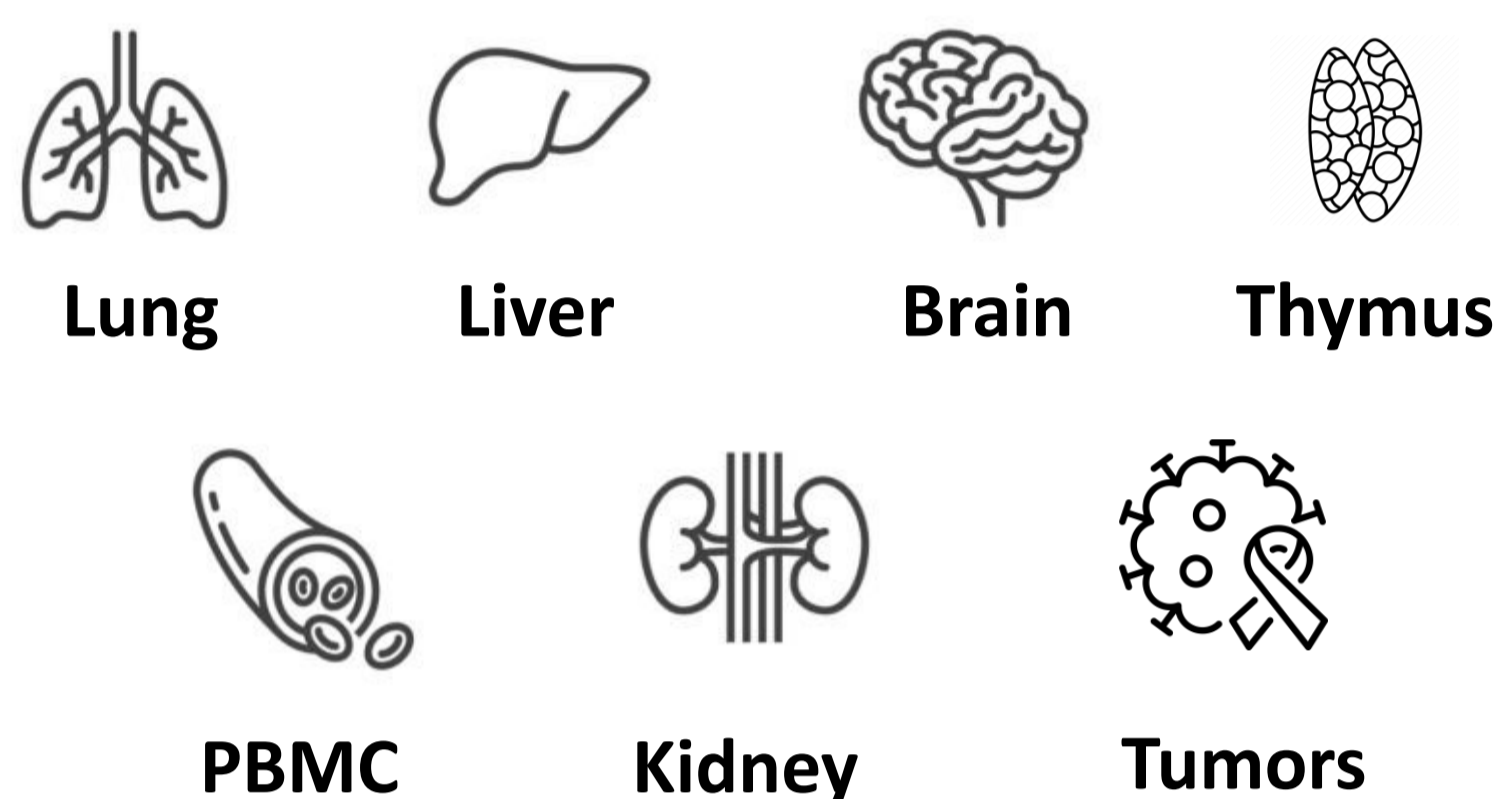
Single-nucleus sequencing offers numerous advantages for analyzing tissues that are difficult to dissociate. It enables the analysis of frozen tissues and access to complex cell populations that are challenging to reach with standard dissociation protocols, thereby revealing cell populations not detected by single-cell sequencing. Additionally, this method reduces cellular stress responses caused by tissue dissociation, preserving the fidelity of the transcriptome.

Main protocol

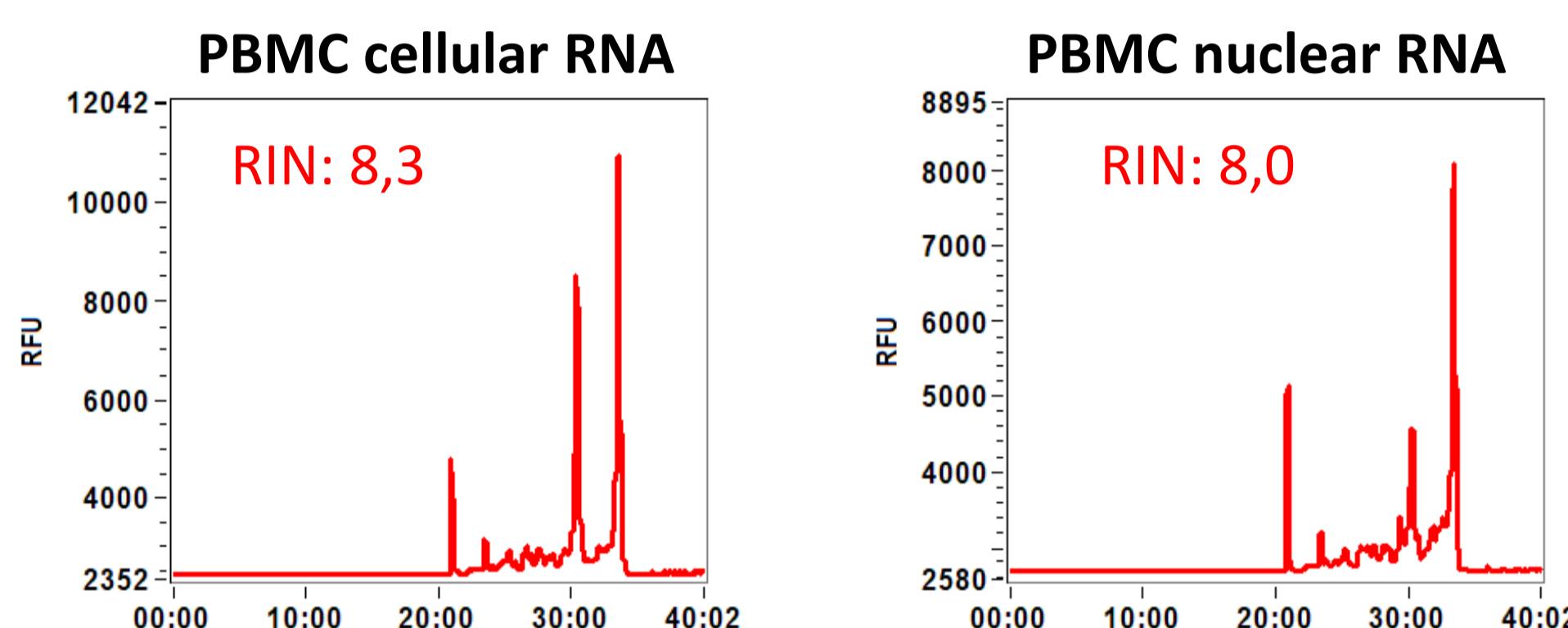
We chose Miltenyi Biotec protocol for its simplicity and adaptability. The process is fast and enables access to clean nuclear suspensions with just one wash (depending on the sample type).



Validated on several types of tissue



Do not affect RNA integrity

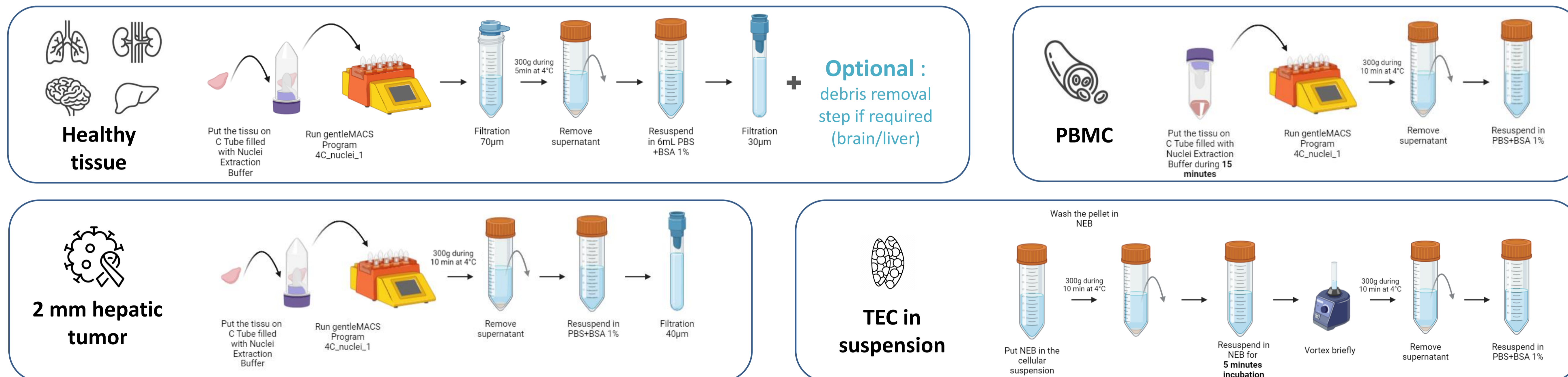


Processes up to 8 samples simultaneously



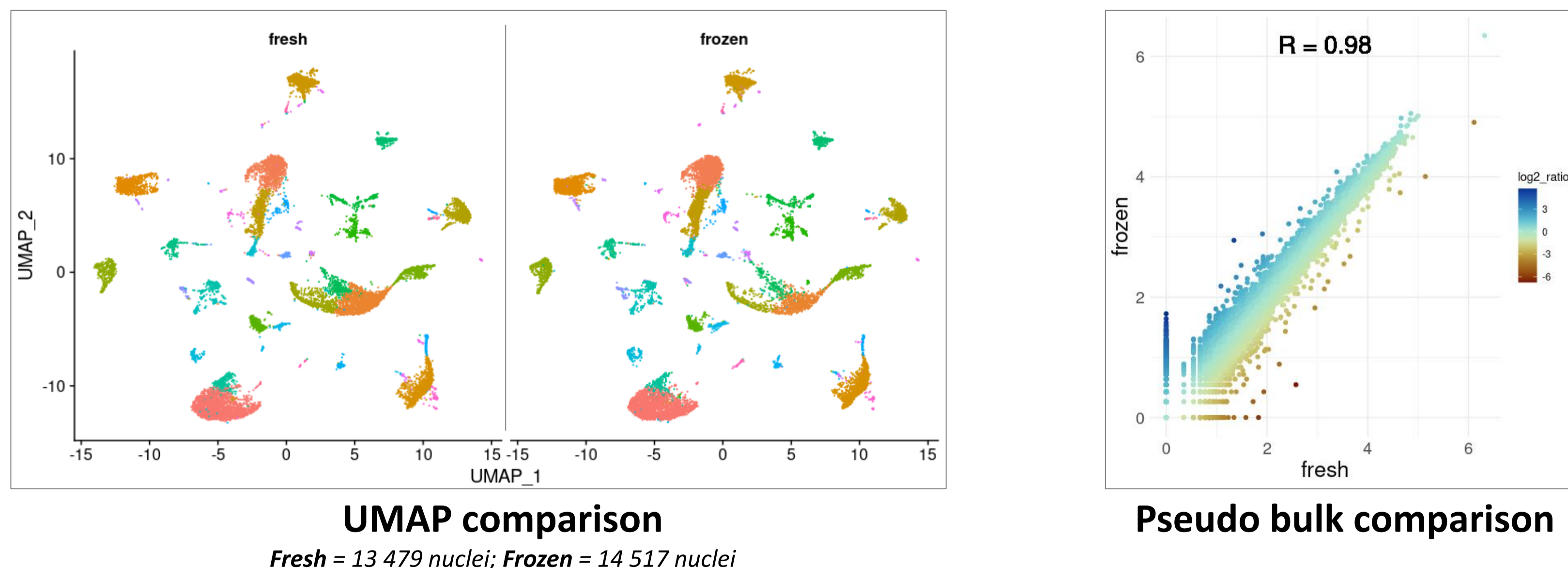
Optimization

Optimization is crucial: adjustments are needed for each tissue type to achieve an improved protocol. Since cells react differently to the nuclei extraction buffer (NEB) — some being more resistant than others — and some samples having a limited number of cells, adapted approaches are necessary.



Nuclei conservation

Freezing is generally not recommended for nuclei as it does not preserve their integrity. However, storage in the **Minute™ Anti-Clumping Nuclei Storage Buffer** by **InventBiotechnologies** appears to be compatible with SnRNAseq.

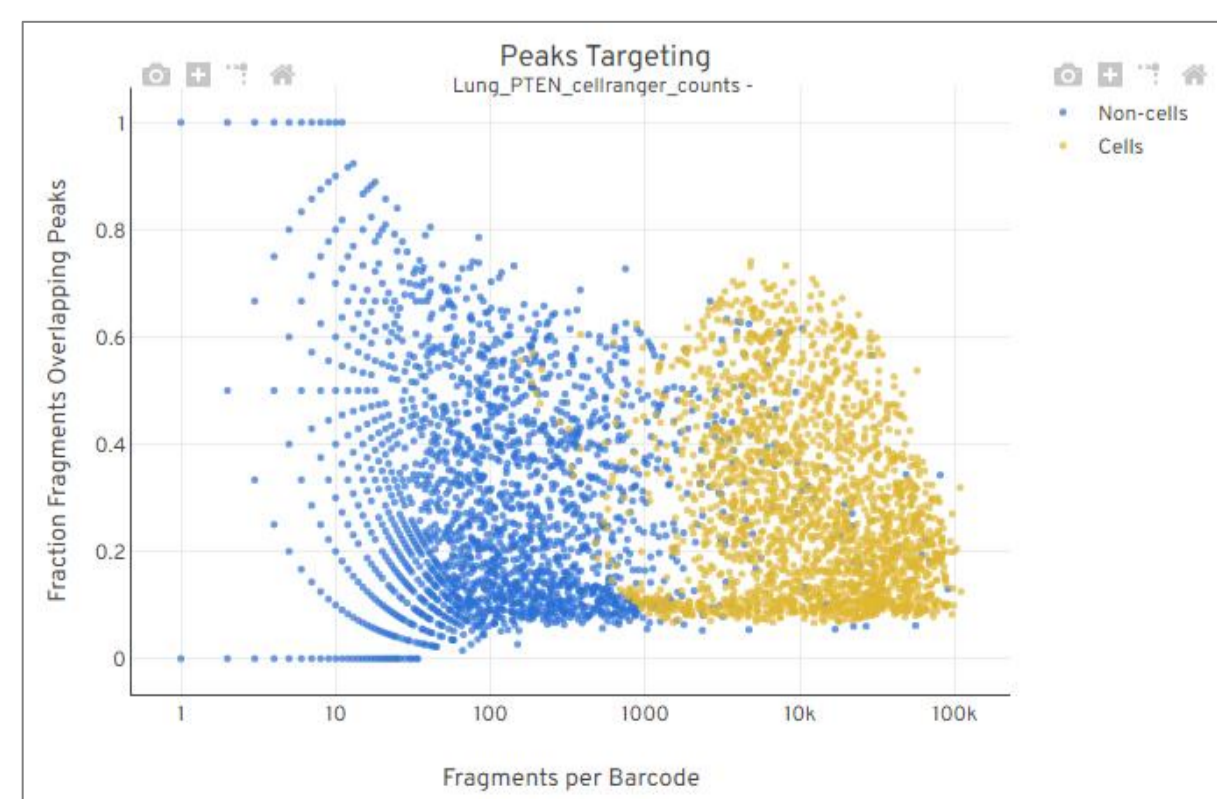


Tested methods

- ✓ Single nucleus RNAseq
- ✓ Single nucleus ATACseq
- ✓ Single nucleus FLEX
- ✗ CellPlex
Not working because the labeling step is too time-consuming, causing the nuclei to start aggregating.

Upcoming project

The upcoming project will investigate the impact of cryopreservation on SnRNA-seq and SnATAC-seq data (Multiomics 10x genomics). By comparing fresh and frozen samples, we aim to assess how cryopreservation influences chromatin structure and gene expression.



We wanted to go that way because we observe poor quality of the chromatin in our data from frozen samples. Indeed, we observe a high number of fragments per barcode but a low percentage of fragments overlapping peaks for the majority of the cells (figure on the left). This raises the question of whether cryopreservation affects chromatin integrity.