

Setting up single nucleus sequencing

Charlyne Gard, Romain Sprynski, Sébastien Nin, Hortense Vachon, Lisa Bargier, Ariadni Liakopoulou, Florent Chuffart, Fayçal Boussouar, Sophie Barral, Saadi Kochbin, et al.

▶ To cite this version:

Charlyne Gard, Romain Sprynski, Sébastien Nin, Hortense Vachon, Lisa Bargier, et al.. Setting up single nucleus sequencing. Assemblée générale 2024 de France Génomique, Jun 2024, Evry courcouronnes, France. hal-04635546

HAL Id: hal-04635546 https://hal.science/hal-04635546v1

Submitted on 4 Jul2024

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Setting up single nucleus sequencing

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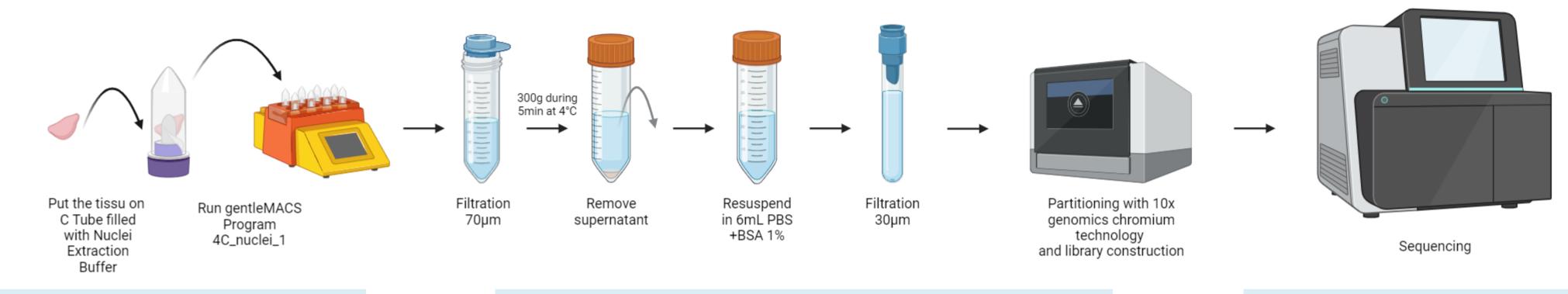


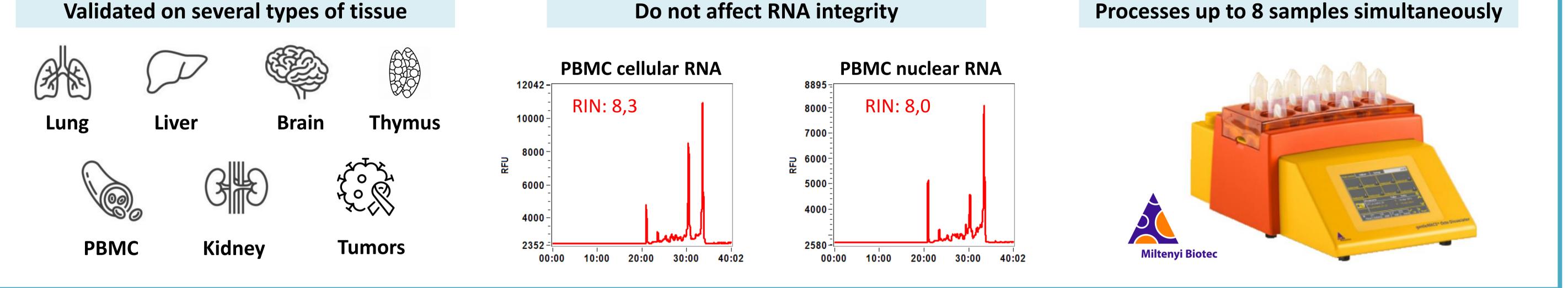
Abstract

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

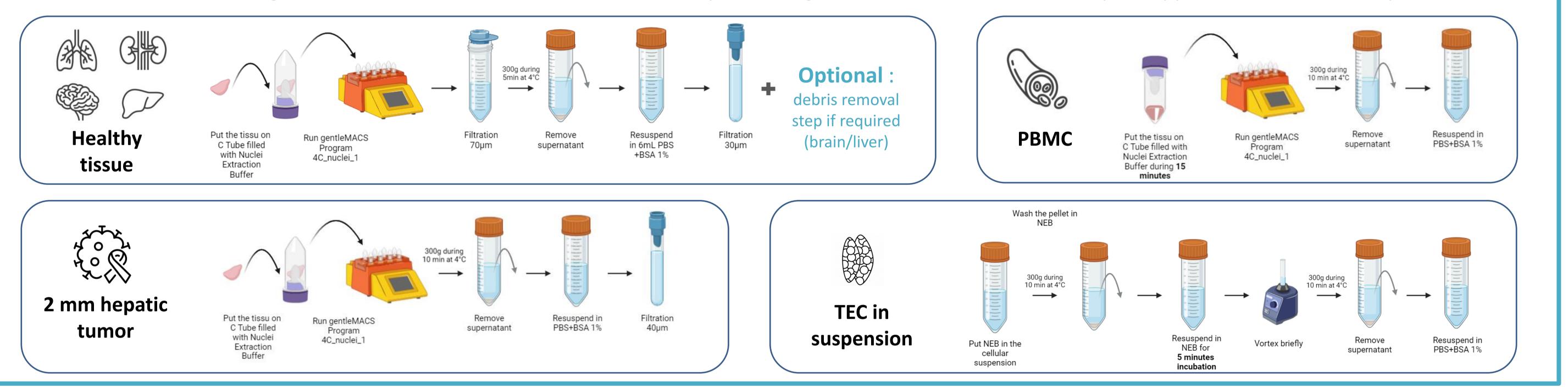
We chose Miltenyi Biotech protocol for its simplicity and adaptability. The process is fast and enables access to clean nuclear suspensions with just one wash (depending on the sample type).





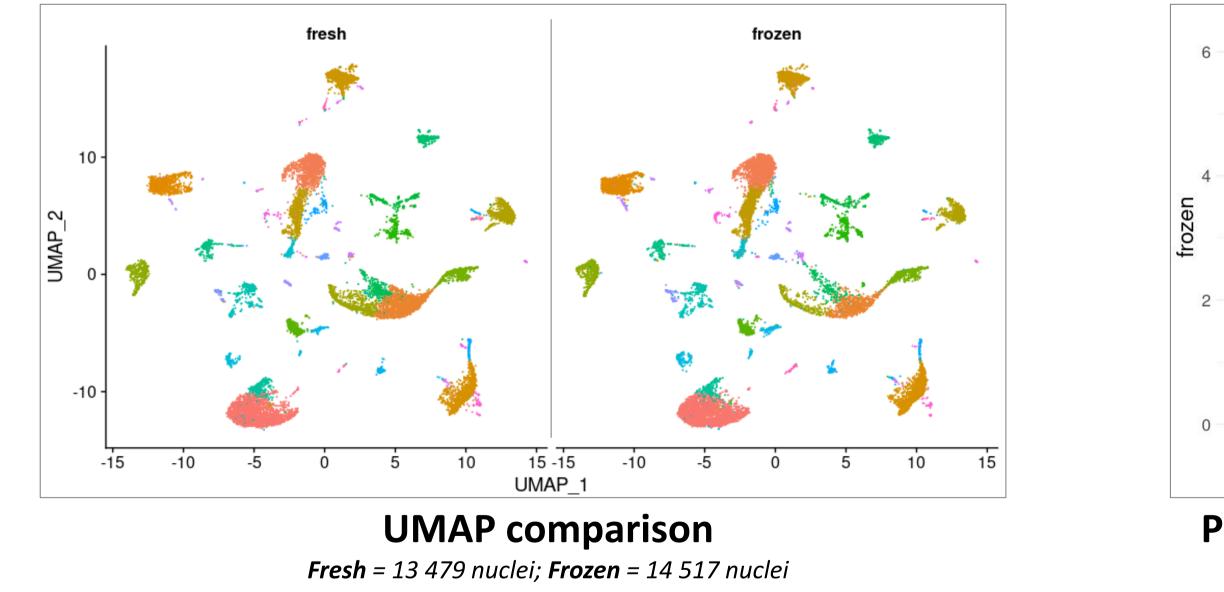
Optimization

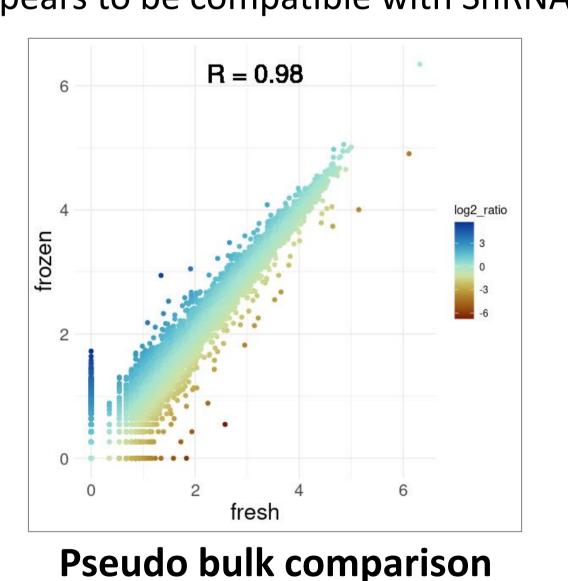
Optimization is crucial: adjustments are needed for each tissue type to achieve an improved protocol. Since cells react differently to the nuclei extraction buffer (NEB) — some being more resistant than others — and some samples having a limited number of cells, adapted approaches are necessary.



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

10× **GENOMICS**

Single nucleus RNAseq

Single nucleus ATACseq

Single nucleus FLEX

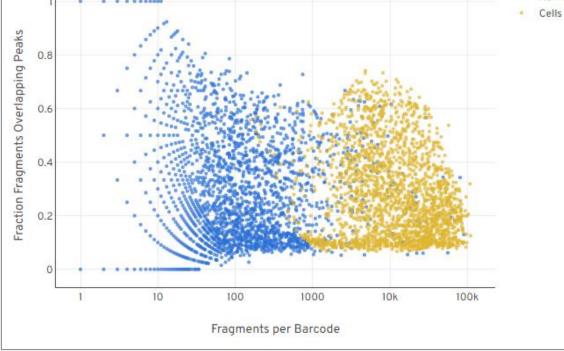
CellPlex

Not working because the labeling step is too time-consuming, causing the nuclei to start aggregating.

Upcoming project

0814 Non-cells

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.



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.



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