Optimizing single nuclei sequencing of brain samples from spaceflown mice across age and strain

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The NASA GeneLab Sample Processing Laboratory offers high-throughput sequencing services to the space biology research community. Space biology studies come with constraints and challenges intrinsic to spaceflight experiments, such as low sample numbers, making the overall study more susceptible to batch effects introduced during sample handling. These issues are compounded by complex protocols such as single-nuclei isolation and sequencing, which has recently become an attractive methodology for assessing the cellular diversity within spaceflight samples.

High quality single-nuclei sequencing requires reproducible protocols to dissociate tissue and generate clean suspension of intact single nuclei. Producing single-nuclei suspension from brain tissue is particularly challenging due to cell type heterogeneity and the myelin sheath that carries over into the nuclei suspension as debris.

Although several methods have been validated by different research groups, these procedures tend to be time consuming and sometimes include steps that can alter gene expression and create cell-type bias. Commercially available nuclei isolation kits, such as the 10x Genomics Nuclei Isolation kit, offers a streamlined way to process samples for nuclei isolation thereby minimizing batch effects and enabling reproducibility.

In this study, we report on the performance of the 10x Genomics nuclei isolation kit and Chromium Next GEM Single Cell Multiome ATAC + Gene Expression kit to generate sequencing libraries from space-flown mouse brain samples. Single nuclei sequencing was performed on frozen mouse brain tissue from two spaceflight missions, Rodent Research-10 (RR-10) and RR Reference Mission-2 (RRRM-2). RR-10 mice were female B6129SF2/J, euthanized at 18-19 weeks whereas RRRM-2 mice were female C57BL/6NTac, euthanized at 20 or 37 weeks.

Sequencing data was processed using GeneLab standard pipelines and the results were used to evaluate the performance of the 10x Genomics nuclei isolation kit for spaceflight samples from mouse brain, and to assess reproducibility across different mouse strains and age groups.