Processing nuclei samples on the BD Rhapsody™ Single-Cell Analysis System

Single nuclei RNA-seq can expand single-cell genomics capabilities

Single nuclei RNA-seq (snRNA-seq) provides a powerful solution for challenges associated with using solid tissues for single-cell genomic research. Obtaining single-cell suspensions from solid tissues requires dissociation methods that may damage cell integrity, particularly cells harboring intricate structures like neurons. This problem can be compounded when using solid tissues preserved by freezing, which can further interfere with cell integrity before downstream single-cell capture methods are employed. However, these issues can be mitigated by using nuclei rather than whole cells for single-cell sequencing. Here, we show how isolated nuclei can be captured and amplified on the BD Rhapsody™ Single-Cell Analysis System. The data demonstrate a high correlation of gene expression patterns in whole cells vs nuclei, as well as successful molecule capture and gene detection in nuclei isolated from previously frozen mouse brain tissue.

BD

Nuclei Isolation

It is recommended that users follow an established nuclei isolation protocol appropriate for the cell and tissue type of their interest.

Modification to the BD Rhapsody System Workflow

Described below are steps in the BD Rhapsody Cartridge protocol, *Single Cell Capture and cDNA Synthesis with the BD Rhapsody Single-Cell Analysis System* (DOC ID210966), that have been modified to accommodate use with isolated nuclei.

Additional reagents needed:

- RNase inhibitor, Murine, 40,000 U/mL (NEB Cat. No. M0314)
- Vybrant® DyeCycle™ Green Stain (Thermo Fisher Scientific Cat. No. V35004)
- Proteinase K, 800 U/mL (NEB Cat. No. P8107S)

Follow *Single Cell Capture and cDNA Synthesis with the BD Rhapsody Single-Cell Analysis System* with the following modifications:

- Before beginning, add 25 µL RNase inhibitor to 5 mL of sample buffer to achieve a final concentration of 0.2 U/µL. Use Sample Buffer containing RNase inhibitor (Sample buffer-RI) throughout the BD Rhapsody Cartridge workflow before lysis.

  Caution: Keep the isolated nuclei within the Sample buffer-RI and on ice or at 4 °C during the process. Use ice-cold buffer for all steps. Avoid unnecessary pipetting.

Counting cells

In the protocol section, *Staining cells with viability markers,* nuclei will be stained with DyeCycle Green rather than Calcein/DRAQ7™.

1. Resuspend the isolated nuclei in 620 µL of Sample buffer-RI.
2. Add 2.6 µL of 5 mM DyeCycle Green (1:250 dilution) and keep on ice for 5 minutes.
3. Proceed to step 6 of the protocol section.

Note: The BD Rhapsody System will read 100% viability since all nuclei will be positive for DyeCycle Green. This does not reflect the true viability of nuclei loaded but the counts generated are accurate.

Bead loading

In the protocol sections, *Preparing cell capture beads* and *Loading and washing cell capture beads*, use Sample buffer-RI instead of Sample buffer.

Nuclei lysis

In the protocol section, *Lysing cells*, follow the recommended modifications:

1. After step 2, remove 1 mL of lysis buffer with DTT from the tube and transfer to a new Eppendorf Tube®. Add 50 µL of Proteinase K. Mix well and put on ice.
2. At step 6, load the cartridge with lysis buffer + DTT containing Proteinase K.
3. At step 7, incubate the cartridge at room temperature for 5 minutes.

Note: The BD Rhapsody System will read 100% viability since all nuclei will be positive for DyeCycle Green. This does not reflect the true viability of nuclei loaded, but the counts generated are accurate. The cartridge metrics from the BD Rhapsody Scanner should be interpreted accordingly.

Continue with BD Rhapsody Library preparation and sequencing with no additional changes.

Figure 1A

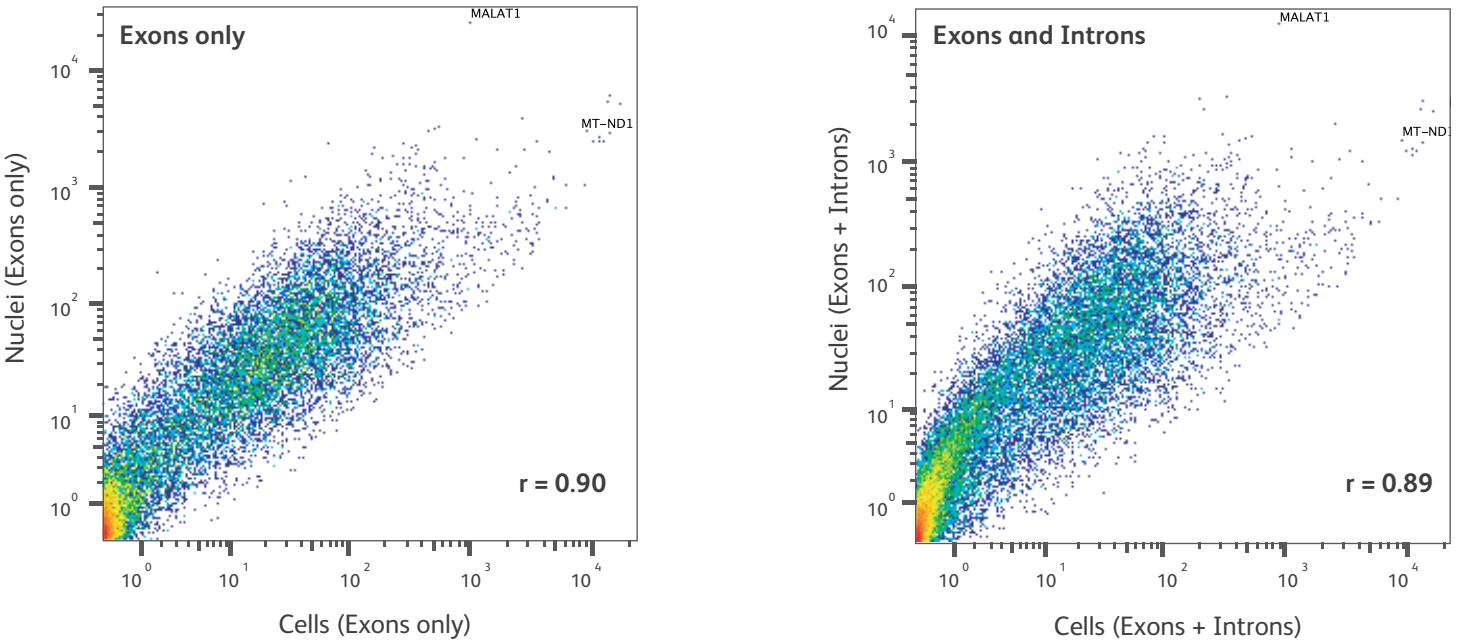

Figure 1B

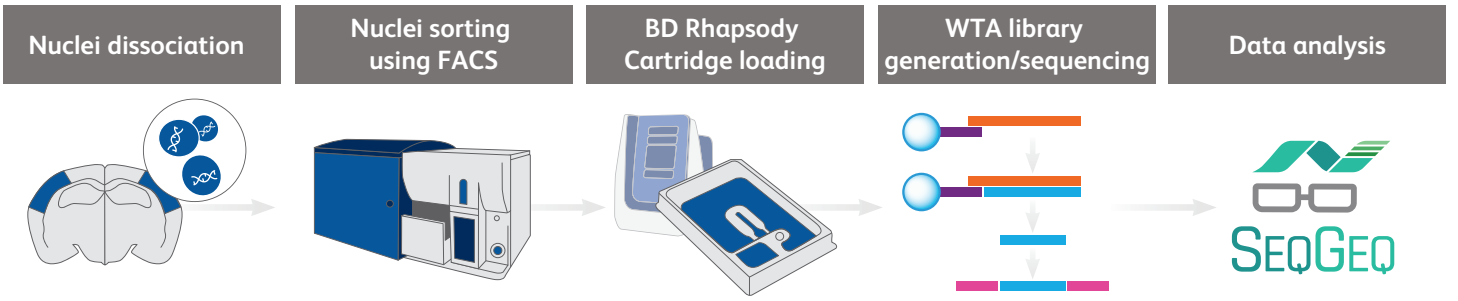

Figure 1C

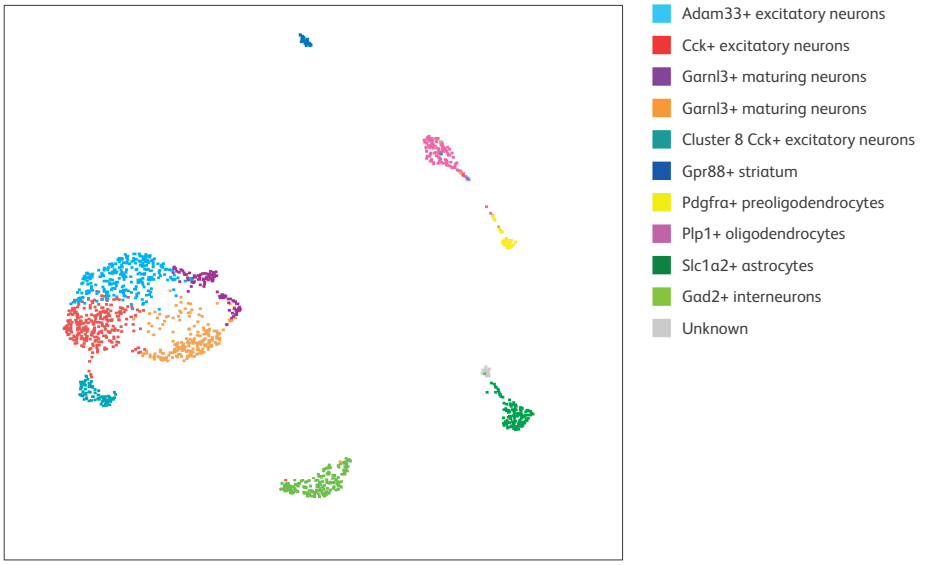

Figure 1. Strong correlation of gene expression between nuclei and whole cells and successful capture of nuclei from dissociated frozen tissue
A. Here, 6,000 cells or nuclei were isolated from Jurkat and Ramos cells (modified isolation protocol[1]) and processed using the BD Rhapsody WTA Kit workflow. Whole transcriptome gene expression based on exons only or exons and introns was then compared between isolated nuclei and whole cells and found to be highly correlated, Pearson's coefficient r = 0.90 and r = 0.89, respectively. **B.** To examine the feasibility on archived frozen tissue samples, nuclei were isolated from C57/BL6 mouse brain (cerebral cortex) (modified isolation protocol[2]). Nuclei were stained with DAPI (1:1,000) and DyeCycle Green (1:250) and sorted for double positive DAPI and DyeCycle Green staining using the BD FACSAria™ III Cell Sorter. The nuclei were loaded onto a BD Rhapsody Cartridge and processed using the BD Rhapsody WTA Kit workflow, sequenced and analyzed using SeqGeq™ v1.6 Software. **C.** Automated cell clustering and annotation using the Seurat plugin for SeqGeq Software showed 11 different cell types with cell composition similar to the published mouse cortex snRNA-seq data in the Allen Brain Atlas (https://portal.brain-map.org/).

References

1 Poglitsch M, Katholnig K, Säemann MD, Weichhart T. Rapid isolation of nuclei from living immune cells by a single centrifugation through a multifunctional lysis gradient. *J Immunol Methods*. 2011;373*(1-2)*:167-73. doi: 10.1016/j.jim.2011.08.012

2 Lake BB, Chen S, Sos BC, et al. Integrative single-cell analysis of transcriptional and epigenetic states in the human adult brain. *Nat Biotechnol*. 2018;36*(1)*:70-80. doi: 10.1038/nbt.4038

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