LeviPrep Nuclei Kit - Raising the Bar for snRNA-seq

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Abstract:

Levitation Technology™ has become an invaluable tool for scientists working with live cells by simplifying workflows, enabling the processing of challenging samples, and by improving a diverse spectrum of costly downstream applications. This same technology can now be used to extract and enrich for nuclei with the release of the LeviPrep™ Nuclei Kit, which is compatible with both the LeviCell® 1.0 and EOS instruments.

LeviCell EOS and LeviCell 1.0 Instruments

Enriching for high-quality nuclei can be challenging, and despite considerable efforts to improve this process, many of the current methods introduce significant variation, require technical expertise, and are not suitable when starting with a low number of cells. Here we introduce the LeviPrep Nuclei Kit, a simple to use solution for nuclei extraction and enrichment. Below we present an outline of the workflow, nuclei quality control steps, sequencing metrics and analysis of nuclei isolated by the LeviPrep method.

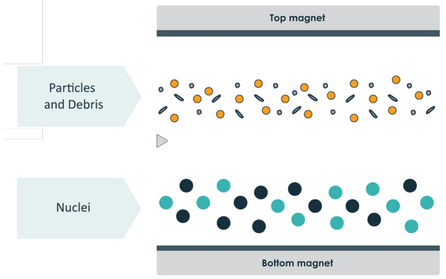


Figure 1: Nuclei Levitation Basics

Particles resuspended in a paramagnetic fluid (Levitation Agent) levitate in the magnetic field created within the LeviCell cartridge according to their unique properties of density and inherent magnetism. The nuclei are enriched and purified from debris and ambient nucleic acid then collected from the bottom channel of the LeviCell cartridge in a simple workflow.

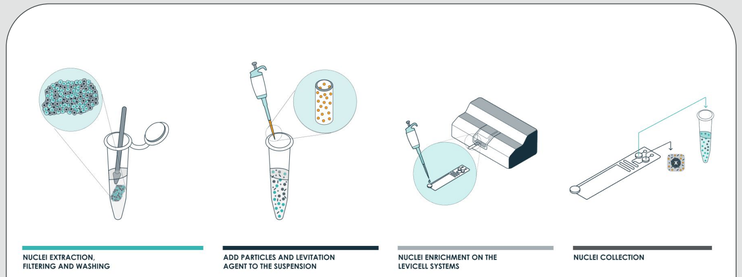
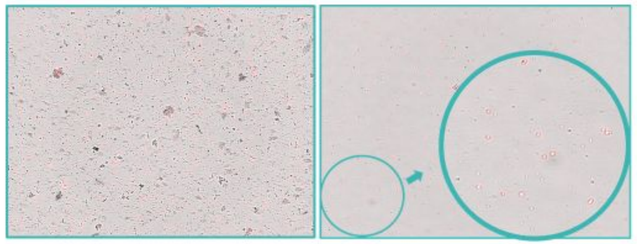


Figure 2: LeviPrep Protocol

Fresh or thawed tissue is gently ground in a microfuge tube with a pestle in the presence of nuclei isolation buffer. The nuclei are filtered, gently centrifuged and the nuclei pellet is washed. The nuclei are then mixed with levitation buffer containing a proprietary particle that removes debris, and processed on the LeviCell 1.0 or LeviCell EOS instrument. This results in high-quality, unlabeled nuclei that are collected from the cartridge, ready for downstream counting, QC, and sequencing.



Initial Sample Post-Levitation Sample

Figure 3: Nuclei QC on an automatic cell counter

Isolated and enriched nuclei are counted on a Nexcelom™ Spectrum (Revvity) instrument. A small aliquot of the output sample is diluted 1:5 in PI/wash buffer and introduced to the Nexcelom instrument. Nuclei appear round and crisp without indication of clumps or blebbing.

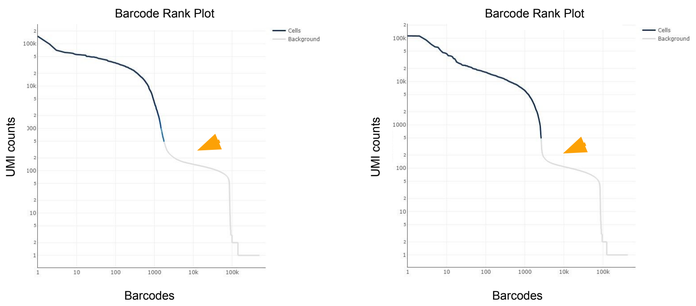


Figure 4: Cell Ranger™ Knee-Plots

Eight frozen mouse brain samples (four each of approximately 10 mg and 100 mg) were prepared using the LeviPrep Nuclei Kit. The nuclei were counted and introduced to the 10X Genomics® Chromium Next GEM Single Cell 3' v3.1 kit targeting 2500 nuclei for each replicate. Libraries were subsequently sequenced on a single lane of NovaSeq X plus™ (Illumina) and FASTQ files were processed through Cell Ranger Cloud. Example knee plots generated by Cell Ranger from 10mg (Left) and 100mg (Right) samples of mouse brain indicate low background RNA contamination (golden arrows) in the resultant nuclei preps.

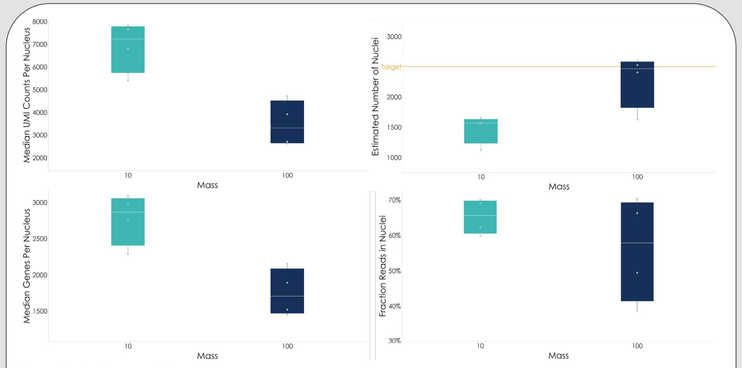


Figure 5: CellRanger QC metrics

Output data from the CellRanger QC summary are presented here. (Top, Left) UMI per nucleus, (Bottom, Left) genes per nucleus and (Bottom, Right) fraction of reads in nuclei are all indicative of high-quality samples (n=4 each mass input represented). (Top, Right) The low deviation of estimated nuclei identified from the target number (goldenrod line, 2500 nuclei) is indicative of accurate QC and low background RNA contamination.

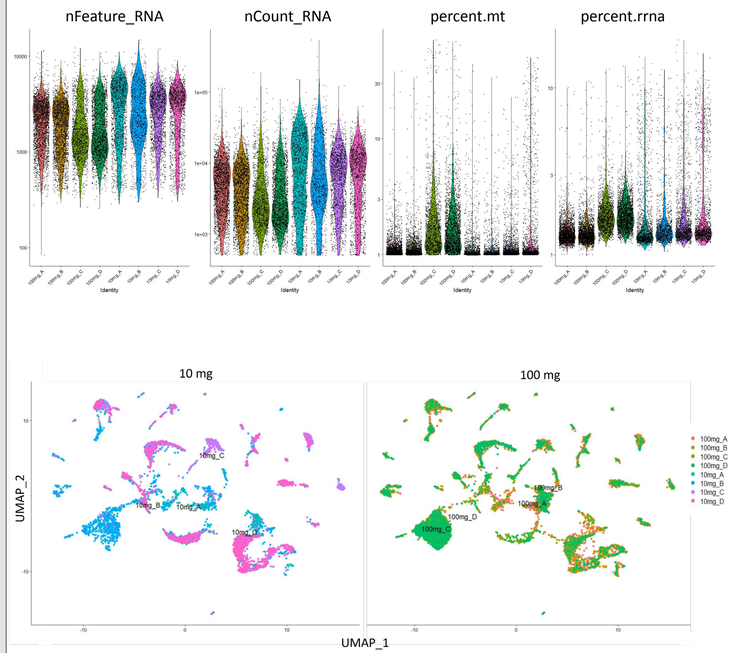


Figure 6: Seurat QC data and sample reproducibility

Cell Ranger outputs including the filtered feature barcode matrices were analyzed using the Seurat software suite. The four replicates of each tissue mass input were analyzed for gene and UMI counts as well as the percent mitochondrial RNA and ribosomal RNA content. Low mitochondrial content indicates high-quality samples and low background ambient RNA. UMAPs drawn comparing the replicates between mass inputs indicate strong similarity between samples.

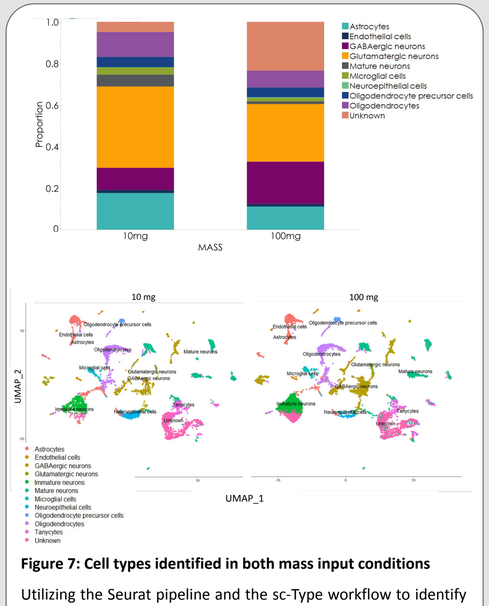


Figure 7: Cell types identified in both mass input conditions

Utilizing the Seurat pipeline and the sc-Type workflow to identify cell types within the brain sample mass groups; we found that regardless of input mass, all the expected cell types within the adult mouse brain were identified in appropriate proportions. Cell types identified include several types of neurons, oligodendrocytes, astrocytes and microglia. UMAPs indicate that either input mass range could be used to identify and study a range of cell types from mouse brain.

Discussion:

Here we describe how the LeviPrep Nuclei Kit successfully extracts and enriches for high-quality nuclei from previously frozen mouse brain tissues for downstream analysis using 10x Genomics Next GEM 3'RNA sequencing. Visual assessment of of nuclei morphology indicates the method is gentle and effective, removing debris and aggregates from the sample without damaging the nuclei. QC metric summaries from Cell Ranger indicate that key indicators of successful sequencing are met or exceeded. Deeper analysis of the sequencing outputs using Seurat indicates that the LeviPrep method is not only easy but reproducible. Lastly, nuclei enriched through the LeviPrep method are unbiased and allow for a full atlasing of cell types from a given tissue from a range of input mass.

References:

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Acknowledgements:
These authors contributed equally to this work*