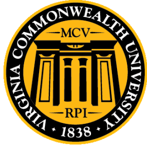

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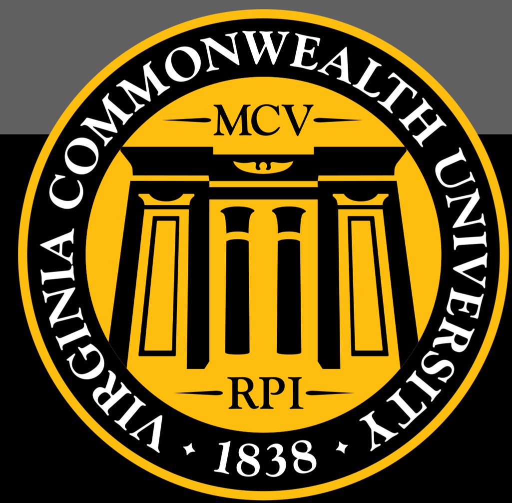

Post-Mortem Brain Nuclei Isolation for Single Nucleus RNA Sequencing

School of Pharmacy | Center for Biomarker Research & Precision Medicine

Charles J. Tran, Lin Y. Xie, Min Zhao, Edwin JCG van den Oord, & Karolina A. Aberg

AIMS

Our objective is to isolate nuclei from frozen human post-mortem brain tissue to study RNA expression profiles using single-nuclei RNA sequencing (snRNA-seq).

BACKGROUND

[Bulk Tissue Challenges] When tissue samples are studied in bulk without consideration for different cell proportions and types, results can be biased due to the attenuation of unique cellular expressions. In order to study cell type specific RNA expression profiles within tissue, single cell RNA sequencing (scRNA-seq) is used[1-2].

[Single Nuclei Sequencing] For scRNA-seq studies it is critical to have intact cells. However, when investigating frozen post-mortem brain tissue, it is often challenging to isolate intact whole cells. An alternative possible solution is to instead isolate nuclei (which have similar, but not identical, transcriptomes to cells) and then perform single-nucleus RNA sequencing (snRNA-seq)[3-4].

METHODOLOGY

[Tissue Homogenization] Working buffers are first prepared from stock solutions according to protocol[5]. Homogenization buffer is added to the post-mortem brain tissue in a tube and rested on ice. This step is important to prevent further damage to the sample caused by rapid temperature change. The sample is homogenized on ice in Homogenization Buffer (HMZB) using a Dounce homogenizer—this is done 20 times with the loose pestle, and 15 times with the tight pestle **(Figure 1).** The volume is then brought up with 2 ml of HMZB using a pipet—the pestle is simultaneously pipet-washed, allowing for the HMZB to run in to the dounce mortar to retain as much sample as possible.

Figure 1. Wheaton Dounce homogenizers (1ml)

[Nuclei Isolation] The volume is brought up to 5 ml with lysis buffer. Filtration of the homogenate is done using a 70 µm strainer **(Figure 2A)**, which further removes cell debris and decreases clumping of nuclei. The solution is then passed through 30 µm strainers twice. The volume is brought up to 10 ml with HMZB then pelleted by centrifugation in 4ºC at 900 x G for 10 minutes. Supernatant is discarded in 10% bleach to eliminate biohazard risks. Pellet is resuspended in 3 ml blocking buffer (containing Rnasin plus and BSA) in order to inhibit any Rnase and the degradation of RNA. 30 µl myelin removal beads are added per 3 ml homogenate. Sample is then incubated in 4ºC for 15 minutes. 3 ml of blocking buffer is added, and pipet mixed until homogenous. Sample is centrifuged in 4ºC at 300 x G for 5 minutes. Supernatant is removed and 3 ml of clean blocking buffer is added **(Figure 2B)**. Pellet is then resuspended. Tube is placed on the magnet stand for 15 minutes in 4ºC and the supernatant is removed. It is vital to avoid contact with sides of the tube closest to magnets in order to avoid resuspending the myelin bounded beads and causing impurity in the sample **(Figure 2C).**

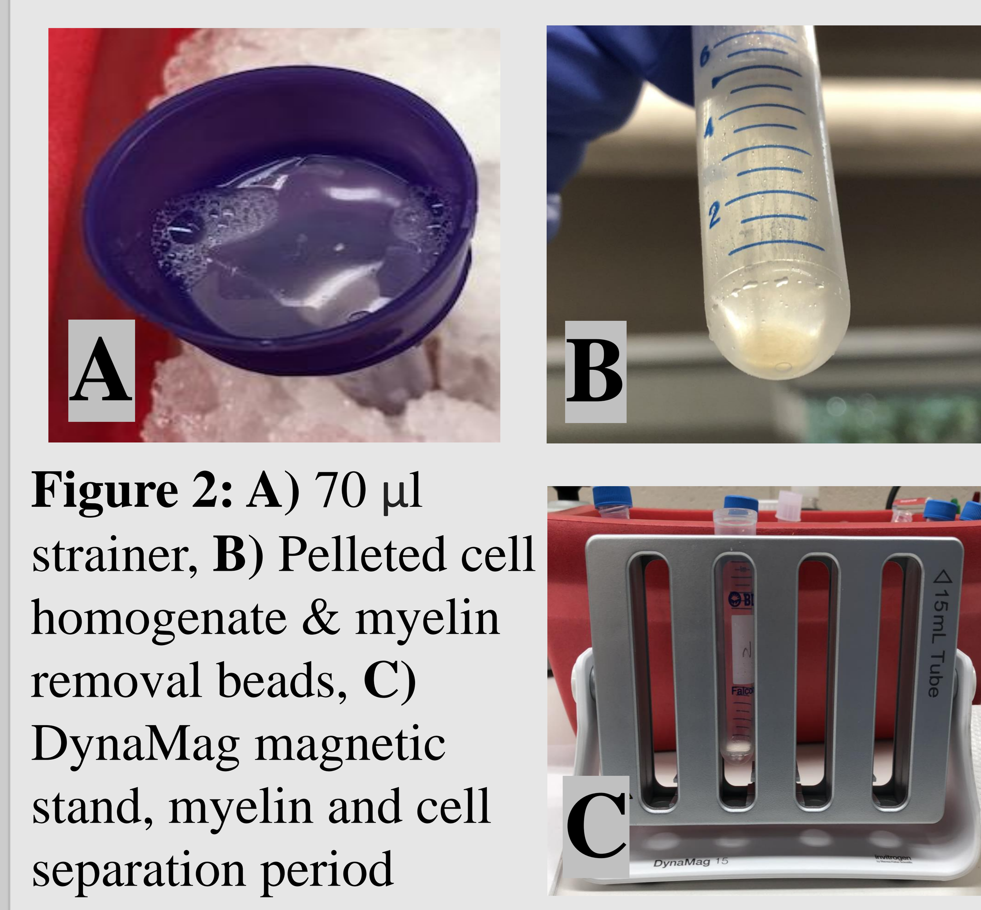

Figure 2: A) 70 µl strainer, **B)** Pelleted cell homogenate & myelin removal beads, **C)** DynaMag magnetic stand, myelin and cell separation period

[Nuclei Quantitation] Using a hemocytometer, the nuclei concentration is measured to determine if the desired number of nuclei (1000/sample) is available for for the downstream sequencing analysis **(Figure 3)**. DAPI dye allows for the identification of nuclei in a mixture of debris.

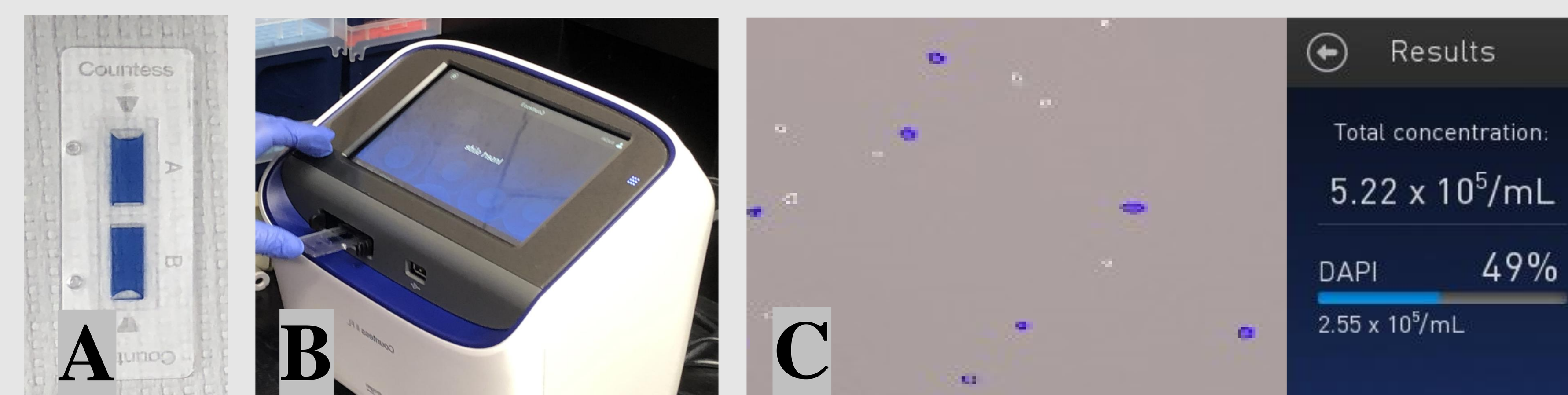

Figure 3: A) Capturing chip, **B)** Countess II Hemocytometer, **C)** DAPI Dyed Nuclei (purple) Concentration Results

[Qualitative Assessment] Microscopy with a 60x oil objective is performed in order to evaluate the integrity of the nuclei after isolation **(Figure 4).** This assessment aids in determining whether the sample is of enough quality or will yield undesirable sequencing results.

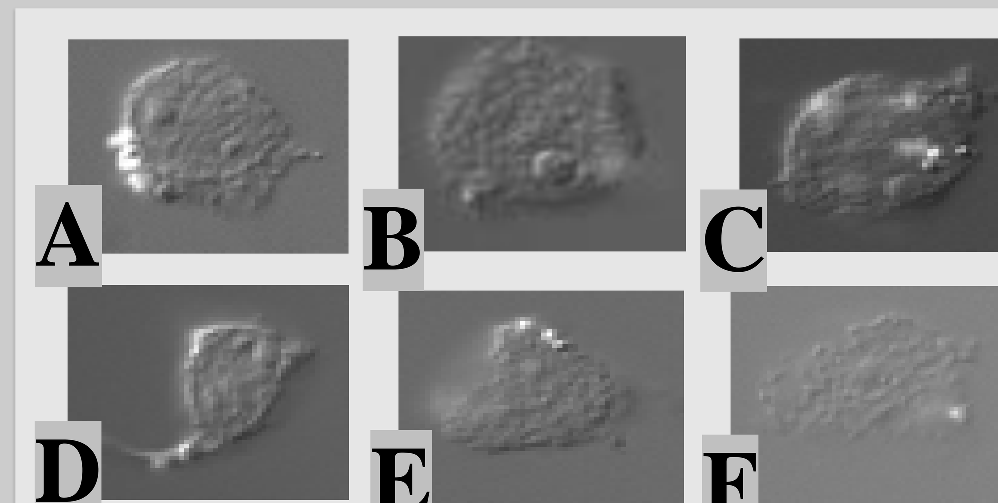

Figure 4: A-C) (Semi) intact nucleus, **D-F)** Non-intact nucleus

RESULTS/DISCUSSION

Using our original protocol, the concentration of nuclei extracted was too low and too many nuclei were broken. Therefore, we optimized the Tissue Homogenization process by adjusting douncing stroke quantity—the tight pestle was found to be extremely harsh on tissue samples and twenty strokes was reduced to fifteen. We then optimized the Nuclei Isolation portion of the protocol by altering strainer sizes and incorporating additional lab techniques such as: (1) adding solution through strainers when bringing up the volume, and (2) simultaneously rinsing pestles while bringing up volume to retain the total sample.

With our optimized protocol, we isolated enough high-quality nuclei from frozen human post-mortem brain samples that are suitable for downstream snRNA-seq analysis.

CONCLUSION

In conclusion we have successfully optimized the protocol for single nuclei isolation from human postmortem tissue that is suitable for downstream snRNA-seq.

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