Quick, Gentle Mammalian Nuclei Preparation and Sorting with the WOLF Cell Sorter

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Introduction

Tissue dissociation methods can cause significant amounts of cell death and alter the transcriptome. Furthermore, attaining a sample that is of sufficient quality for a sequencing experiment can be almost impossible when working with tissue samples. Using nuclei instead of intact cells has become a growing method for downstream next-generation sequencing applications, single nucleus RNA-Seq (snRNA-seq). Using nuclei expands the capability to work with preserved samples and cell types that are challenging to obtain a single cell suspension from such as neurons. However, nuclei isolation methods often leave a significant amount of debris in the suspension that can compromise the results. Therefore, debris removal is a critical sample preparation step in generating robust sequencing results. One common way to clean up your sample is by using a cell sorter, however traditional cell sorters that use higher pressure sorting mechanism can damage nuclei. The WOLF® Cell Sorter is gentle, microfluidic cell sorter that is capable of efficiently removing unwanted particles from your sample without causing shear stress.

In this application note, we partnered with Invent Biotechnologies Inc. and Biostatus to develop a quick and gentle method for isolating and sorting mammalian nuclei.
Nuclei isolation kits used in this study employ a proprietary filter cartridge-based technology and anti-aggregate buffer systems for rapid isolation of single nuclei from fresh or frozen tissues. The kits are simple and easy to use. The whole protocol can be completed in less than 30 min.

Method

Human embryonic kidney 293 (HEK293) cells and Mouse brain tissue (Rockland) were processed to a nuclei suspension using the Invent Biotechnologies Minute™ Detergent-Free Nuclei Isolation Kit (#NI-024) and Minute™ Single Nucleus Isolation Kit for Neuronal Tissues/Cells (#BN-020) respectively. Nuclei were then confirmed using Trypan blue on the Countess™ II Automated Cell Counter (Figure 2A). The nuclei cell suspension was then diluted to 200-300 events/uL with PBS + .1% BSA and filtered through a 37um filter. Nuclei were then stained with DRAQ7 DROP & GO™ at 2 drops per 0.5ml and incubated on ice for 10mins. An unstained nuclei sample was used for a negative control. Nuclei suspensions were then analyzed and sorted on the WOLF. Sort gates were set to exclude debris by gating out small particles and DRAQ7 negative events (Figures 2-3).

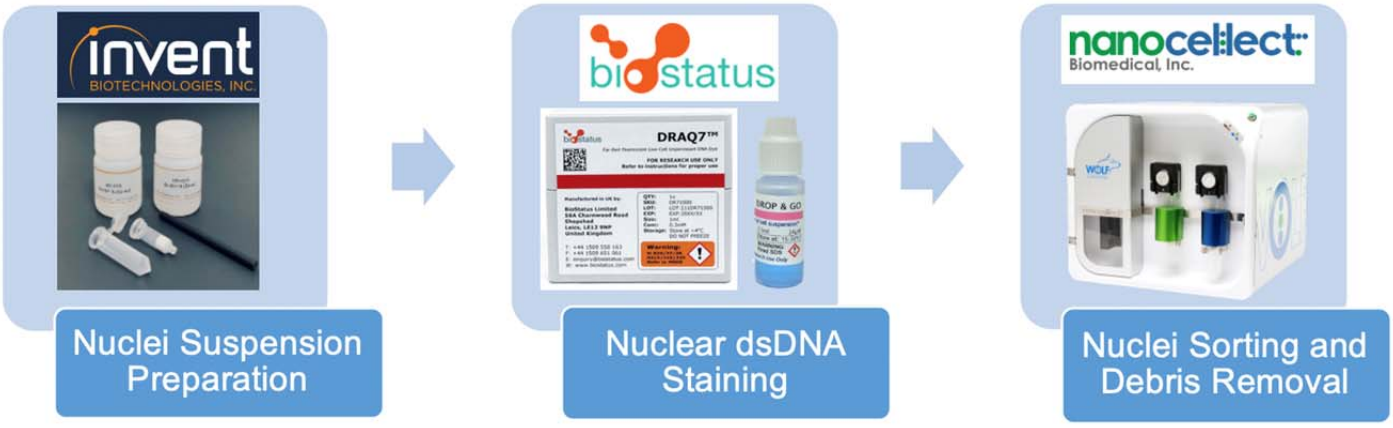

Figure 1. Workflow Overview. Cells and tissues were processed into a nuclei suspension by using the Invent Biotechnologies nuclei isolation kits. Nuclei were then identified using the Biostatus DRAQ7 DROP & GO. Finally, stained nuclei and identified and sorted on the WOLF.

Results

Nuclei could be identified by looking at the BSC-H/DRAQ7 parameters. Discrete amounts of DNA content could also be identified based on the different intensities of DRAQ7. From the HEK293 nuclei suspension, 35% of the population was identified as nuclei before sorting. After sorting for the DRAQ7+ nuclei population, the amount of debris was reduced by more than half (Figure 2B).

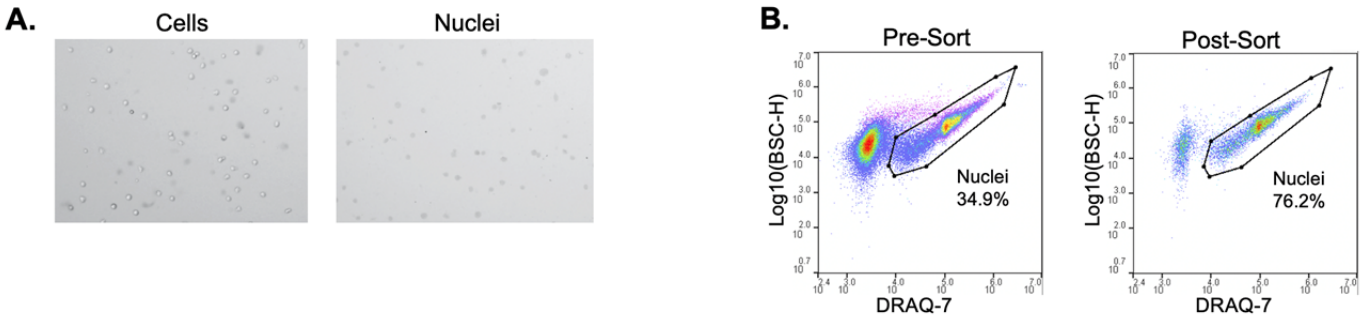

Figure 2. HEK293 Nuclei Sort. (A) Nuclei were confirmed after using the nuclei isolation kit **(B)** Pre-sort and Post-sort percentage of HEK293 nuclei.

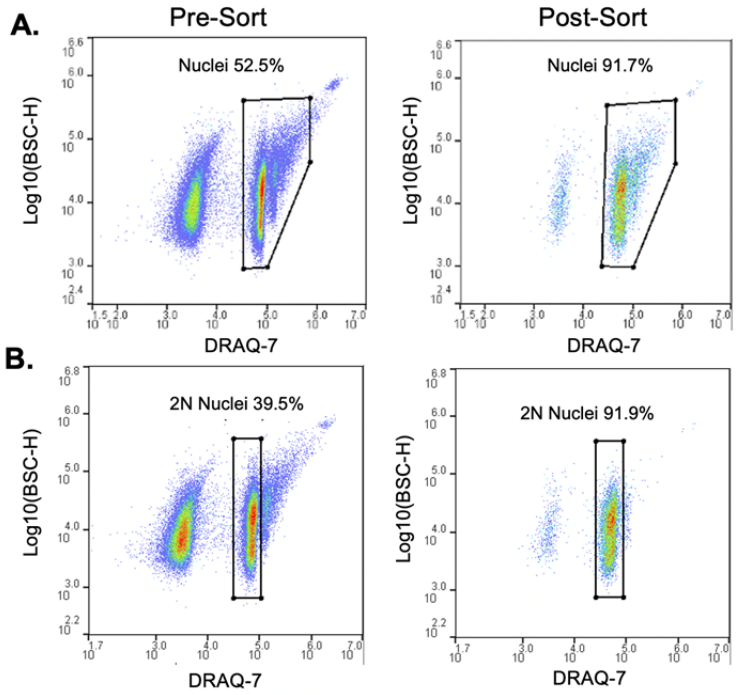

Figure 3. Mouse Brain Nuclei Sort. Pre- and Post-Sort results from a **(A)** total nuclei sort and **(B)** 2N nuclei sort.

Next, mouse brain tissue was processed into a nuclei suspension and stained with DRAQ7 DROP & GO. Pre-Sort analysis showed that half of the sample was nuclei while the other half was contaminating debris. After setting the WOLF to sort for only the DRAQ7+ nuclei population, the WOLF was able to significantly reduce the amount of debris and enrich the nuclei population to around 92%. Furthermore, some research studies may only be interested in a certain type of nuclei, so a 2N nuclei sort was also conducted on the WOLF. Results from this sort also showed an enrichment of 92% 2N nuclei.

Conclusion

By using the Invent Biotechnologies detergent-free nuclei isolation kit, sample preparation only took 30 minutes and the nuclei remained intact. Moreover, no clogs were observed during the sort. Furthermore, successful staining was observed with the Biostatus DRAQ7 DROP & GO an incubation time of only 10 minutes. In conclusion, results from these experiments demonstrate a gentle and quick workflow to isolate and sort nuclei from cells and tissue.