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Application Note

Single Nuclei Dispensing

Introduction

Single-cell RNA-sequencing (scRNA-seq) has been widely adopted for investigating gene expression in individual cells, offering unparalleled resolution into cell type-specific genomic identities. Recently, single nuclei RNA-sequencing (snRNA-seq) has emerged as a valuable alternative to scRNA-seq, allowing researchers to capture mRNA transcripts from individual nuclei. This approach is compatible with frozen cell or tissue samples and additionally reduces sample dissociation biases as well as dissociation-induced transcriptional stress responses.[1] Namocell's single cell dispensers offer a high throughput, benchtop solution to single cell isolation for plate-based genomics applications, such as scRNA-seq. In this application note, a Namocell dispenser was used to isolate single DNA-labeled nuclei to demonstrate compatibility with snRNA-seq workflows (FIGURE. 1).

FIGURE 1. Schematic of Namocell's approach to single nuclei dispensing for plate-based snRNA-seq.

Methods

Intact nuclei were isolated from tissue according to a standard nuclei isolation protocol. Nuclei in suspension were stained using 20nM SYTO™ 16 Green Fluorescent Nucleic Acid Stain (Thermo Fisher Scientific, S7578) for 10 minutes at room temperature, and then diluted to 5,000 nuclei/mL. The diluted nuclei were loaded into a sterile microfluidic cell cartridge (Namocell) and loaded into the Namocell system. Nuclei were sorted using the FITC detection channel (533nm) to identify positively labeled nuclei (with a lower bound of FITC=20). Individual FITC-positive nuclei were dispensed onto a glass slide placed on a 96-well plate.

Results

Single, intact nuclei were verified following dispensing via fluorescence microscopy (FIGURE 2). Overall, Namocell's single cell dispensers provide a simple and gentle (<2 psi) option for easy isolation of nuclei for snRNA-seq

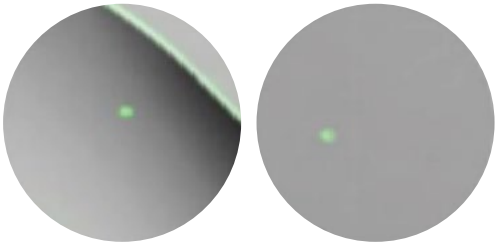

FIGURE 2. Single nuclei dispensed onto a glass slide and visualized with a fluorescent microscope.

References

1. Wu, H., Kirita, Y., Donnelly, E.L., Humphreys B.D. 2019. JASN 30: 23-32.