Fluent BioSciences

APPLICATION NOTE

PIPseq Compatibility with DSP–Methanol Fixation of Cells and Nuclei

Introduction

PIPseq is a highly scalable single-cell RNA sequencing solution that provides cost-effective library preparation for every researcher's project. As the scope of single cell RNA sequencing studies continues to expand, high quality sample input continues to be critical for experimental success. Researchers looking to perform complex studies involving intracellular protein staining, extensive cell sorting and processing, long distance transportation of samples, and extended time course studies are limited by the potential for sample degradation during these processes. Cell or nuclei fixation provides a solution to these challenges, preserving the single cell or nuclei samples for additional or delayed processing. With a simple DSP-methanol fixation procedure researchers with more complex experimental designs can also take advantage of the flexible, user-friendly PIPseq platform for scalable single-cell RNA sequencing.

In this application note, human PBMCs and mouse brain nuclei were processed with and without DSP-methanol fixation with comparable sequencing results, making PIPseq fixation compatibility an advantageous addition to Fluent Biosciences' flexible, scalable, and cost-effective single-cell RNA sequencing solutions.

Methods

A fresh fixation buffer composed of 20 µL of 50 mg/mL DSP ((dithiobis(succinimidyl propionate)), "Lomant's Reagent",) in DMSO in 800 µL 100% chilled MeOH (Sigma-Aldrich, Cat# 34860) was prepared, gently mixed, and kept on ice. 1 million cells or nuclei were resuspended in 200 µL of Fluent Cell or Nuclei Suspension Buffer and 820 µL of chilled fixative was gradually added while swirling. Cells were fixed on ice for 30 minutes, swirling to mix occasionally. Nuclei were fixed on ice for 15 minutes, swirling to mix every 3-4 minutes. After fixation the DSP-methanol mixture was quenched with 20.4 µL 1 M Tris pH 7.5. Fixed cells and nuclei were maintained at –20 ˚C for one week.

In preparation for PIPseq the fixed cells or nuclei were equilibrated on ice. Cells were centrifuged for 5 mins at 500 x g at 4 ˚C and the supernatant was discarded. Cells were then resuspended in ~100 µL of Resuspension Buffer (3X SSC, 1 uM DTT, 0.2 U/µL RNase Inhibitor, 1% BSA by mass in nuclease-free water) at the necessary concentration.

For nuclei for each 1 mL of nuclei in fixative, 2 mL of 1X Nuclei Suspension Buffer was gradually added, swirling between additions. The nuclei were then centrifuged for 3 mins at 500 x g at 4 ˚C, and the supernatant was then discarded. The nuclei pellet was then resuspended in ~100 µL 1X Nuclei Suspension Buffer at the appropriate concentration. The cell or nuclei concentration was quantified, concentration adjusted if necessary, and used as input to the standard PIPseq 3' single cell RNA sequencing pipeline (PIPseq 3' RNA T2 (FB0001026), T20 (FB0002130), or T100 (FB0003657) kits).

Results

Table 1. Comparison of Key Performance Metrics between unfixed and DSP-methanol fixed human PBMCs

		DSP-Methanol	
	UNFIXED	FIXED, 1 WK	% DIFFERENCE
MAPPING RATE	91.5%	88.5%	-3.3%
MEDIAN TRANSCRIPTS / CELL	2541	2020	-20.5%
MEDIAN GENES / CELL	1226	1249	+1.9%

Figure 1: Barcode-Rank Plots comparing unfixed and DSP-methanol fixed human PBMCs

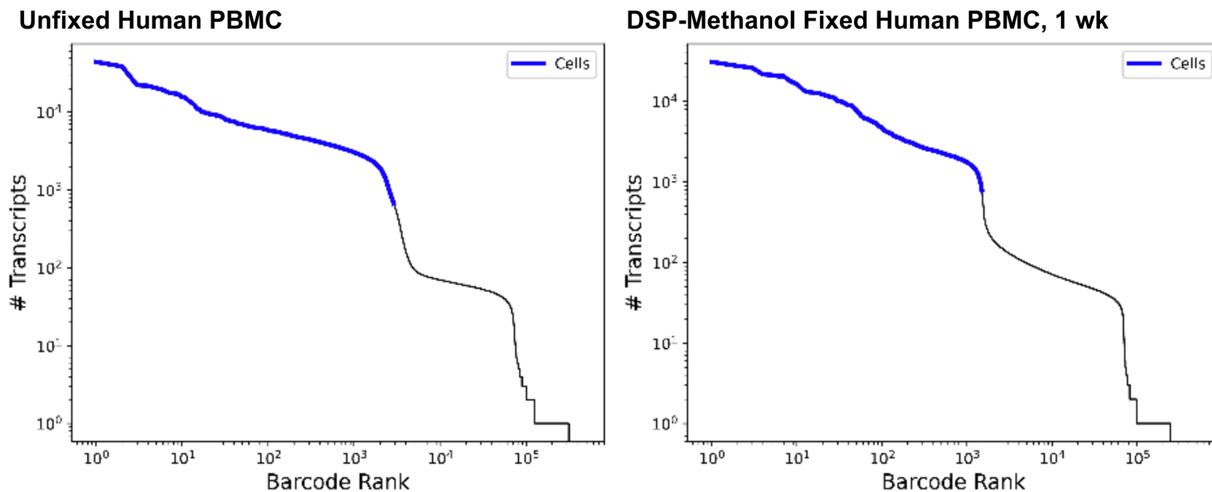


Figure 2: UMAP Plot With Cell Type Annotations comparing unfixed and DSP-methanol fixed human PBMCs

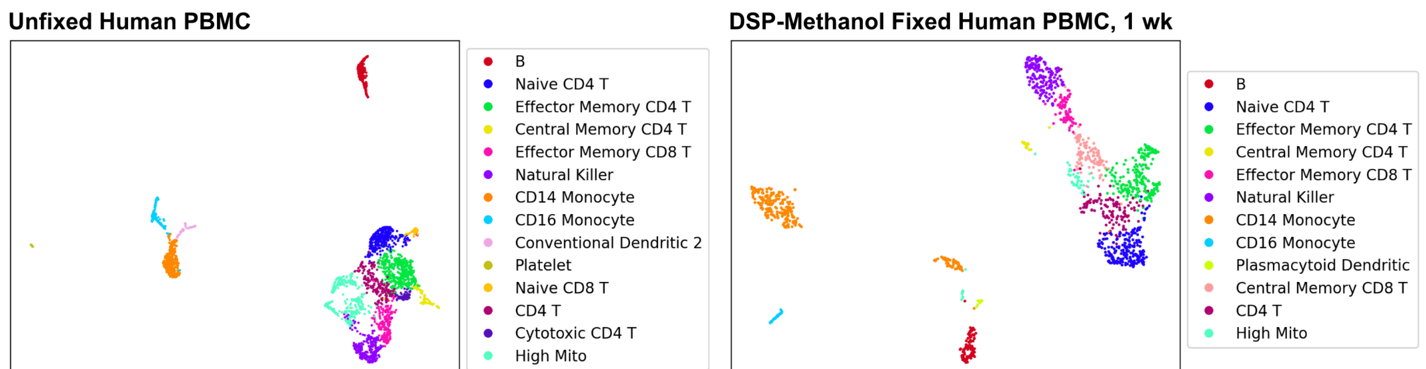


Table 2. Comparison of Key Performance Metrics between unfixed and DSP-methanol fixed mouse brain nuclei

		DSP-Methanol	
	UNFIXED	FIXED, 1 WK	% DIFFERENCE
MAPPING RATE	82.8%	80.8%	-2.3%
MEDIAN TRANSCRIPTS / CELL	3986	4050	+1.6%
MEDIAN GENES / CELL	1802	1865	+3.5%

Figure 3: Barcode-Rank Plots comparing unfixed and DSP-methanol fixed mouse brain nuclei

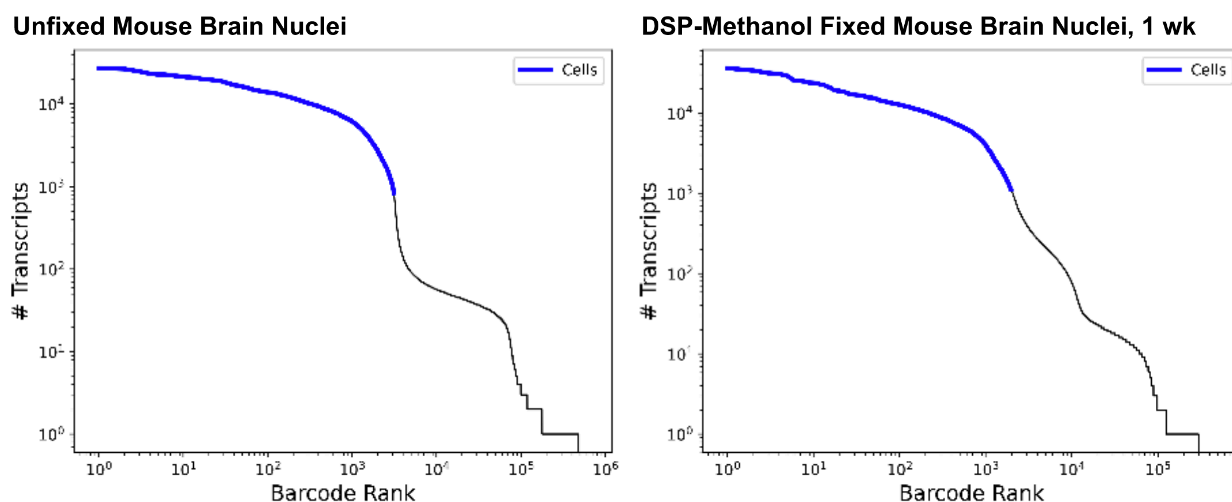
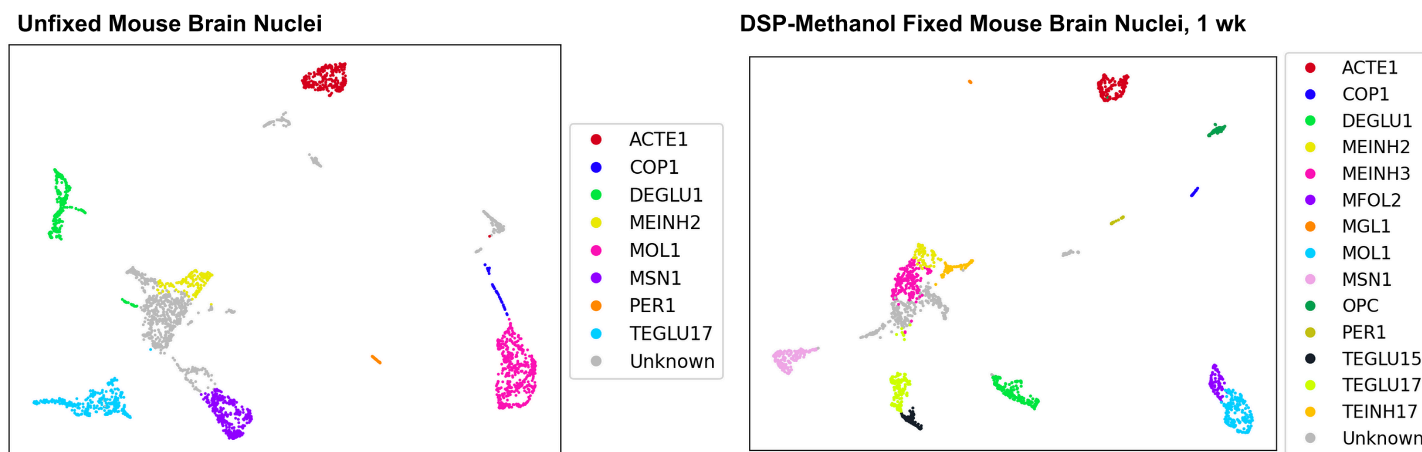


Figure 4: UMAP Plot With Cell Type Annotations comparing unfixed and DSP-methanol fixed mouse brain nuclei



Discussion

Fluent Biosciences has demonstrated that a mixture of DSP ((dithiobis(succinimidyl propionate)), "Lomant's Reagent") and methanol effectively fixes both primary (e.g. PBMC) and cultured cell lines (e.g. HEK/3T3) as well as isolated nuclei and has been validated for compatibility with PIPseq 3' Single Cell RNA v4.0PLUS kits. Unfixed and DSP-methanol fixed cells and nuclei both showed comparable sensitivity metrics, cell quality, clustering, and automated cell type annotation on the PIPseeker platform (Table 1-2, Figure 1-4). In both cells and nuclei DSP-methanol fixation slightly increased the median genes per cell recovered, 1.87% in cells and 3.49% in nuclei, suggestive of transcriptome preservation with fixation (Table 1). There is a negligible difference in the mapping rate between unfixed and fixed cells and nuclei, and a minor drop in median transcripts per cell recovered that does not impact median genes per cell (Table 1-2). The barcode-rank plots presented with comparable trends in both fixed and unfixed cells and nuclei (Figure 1,3). Clustering and cell type annotation was equally comparable with high quality and well separated cell types being identified (Figure 2,4). There was increased cell annotation granularity with DSP-methanol fixed mouse brain nuclei (Figure 4).

Together this data demonstrates PIPseq compatibility with DSP-methanol fixation of cells and nuclei. The straightforward fixation protocol will enable even greater flexibility of sample processing for input to PIPseq, broadening the range of single-cell sequencing data that Fluent Biosciences can provide. Fixation compatibility will directly benefit researchers and genomics cores looking to process samples that require transportation, time course experiments, long processing steps, and more. Overcoming these limitations with fixation compatibility further enables PIPseq researchers to get the highest quality single-cell sequencing results from their experiments.

References

FB0004708: Demonstrated Protocol DSP-Methanol Fixation for Cells

FB0004745: Demonstrated Protocol DSP-Methanol Fixation for Nuclei

FB0001026: PIPseq T2 v4.0PLUS Single Cell RNA Kit User Guide

FB0002130: PIPseq T20 v4.0PLUS Single Cell RNA Kit User Guide

FB0003657: PIPseq T100 v4.0PLUS Single Cell RNA Kit User Guide

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