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APPLICATION NOTE

HIVE scRNAseq Solution: A Case Study Demonstrating Sample Stability & Fragile Cell Recovery

HIVE™ scRNAseq Solution

INTRODUCTION

Biological resolution at the level of individual cells is powering the next phase of precision health. The HIVE™ scRNAseq Solution integrates sample storage and single cell profiling into a single workflow, solving issues associated with the logistics of sample collection across multiple sites. The HIVE™ scRNAseq Solution enables the generation of biologically complete and high-quality data from samples that require storage prior to processing for single-cell RNA libraries.

METHODS

1) Sample Preparation

Commercially available blood from a healthy human donor was delivered same day to the Honeycomb Biotechnologies lab. Red blood cells were depleted by size selection using a sterile Acrodisc® White Blood Cell Syringe Filter (Pall Laboratory, # AP-4951), preserving all leukocyte populations, including granulocytes.

2) Sample Capture

Six HIVE™ devices were each loaded with approximately 15,000 cells in 1 mL of cell media. Single-cells settled into picowells of the HIVE containing 3' transcript-capture beads. Three cell-loaded HIVE™ devices were processed immediately without storage, and three were frozen at -20°C for two weeks after the addition of Cell Preservation Solution, before being processed through to single-cell NGS libraries.

3) HIVE Processing and Library Preparation

Following standard protocol, cell-loaded HIVE devices were sealed with a semi-permeable membrane, allowing for the use of the strong Lysis Solution followed by the addition of Hybridization Solution. After collection with or without storage, beads with captured transcripts were extracted from the HIVE™ device by centrifugation. The remaining HIVE™ library preparation steps were conducted in a 96-well plate format. PerkinElmer's LabChip® GX Touch™ nucleic acid analyzer was used for accurate assessment of individual library sizes, and the concentration of final pooled libraries was determined by qPCR.

4) Sequencing & Analysis

HIVE™ scRNAseq libraries were sequenced using specific primers contained in the kit on an Illumina® NovaSeq® 6000 sequencer. Demultiplexed FASTQ files were input for analysis with the BeeNet™ software solution, which has been specifically designed for the HIVE™ scRNAseq libraries and is provided with the HIVE™ system, outputting count matrix files. Seurat was used for secondary analysis. Cells that fell below a threshold of 400 genes and 800 transcripts identified were excluded. Additional filtering was done to remove low quality cells as indicated by greater than 0.1% mapping to mitochondrial reads and to remove mixed clusters based on co-expression of mutually exclusive lineage markers with the same barcode, which suggests more than one cell settled into the same nanowell.

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RESULTS

The libraries generated from all six HIVE devices were within acceptable concentration (> 1ng/µL) and peak library sizes (500 - 1,200 bp).

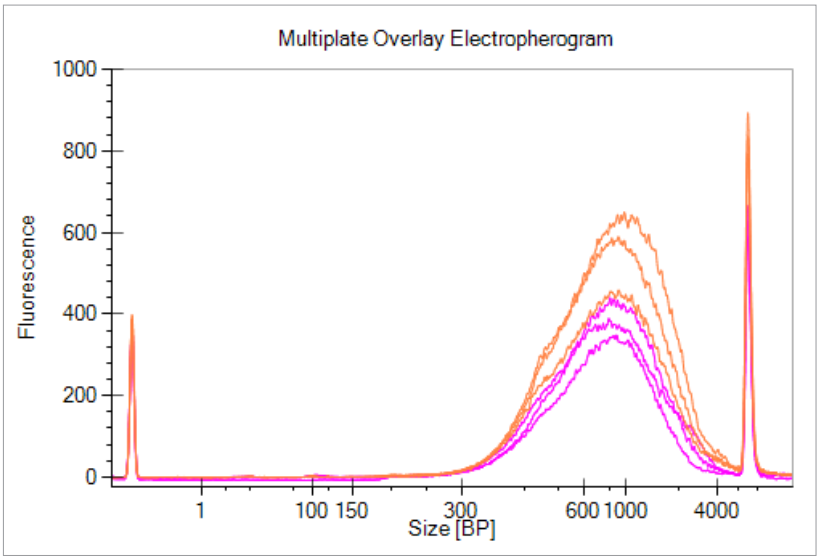

Figure 1: LabChip® electropherogram, orange traces = fresh samples, magenta traces = after the samples were stored @ -20°C for 2 weeks

After thresholding and filtering for high quality single-cells as described above, there were 24,720 cells in total that passed QC from all six HIVE™ devices. Sixteen major cell type clusters were identified, including multiple T cell and B cell, dendritic cell, and monocyte clusters. Multiple clusters corresponding to different granulocyte cell types such as neutrophils, eosinophils, and basophils were identified as well, which are known to be challenging to recover in droplet-based platforms. Additionally, there was robust and specific expression of canonical lineage markers for each cluster, including the granulocyte cell types.

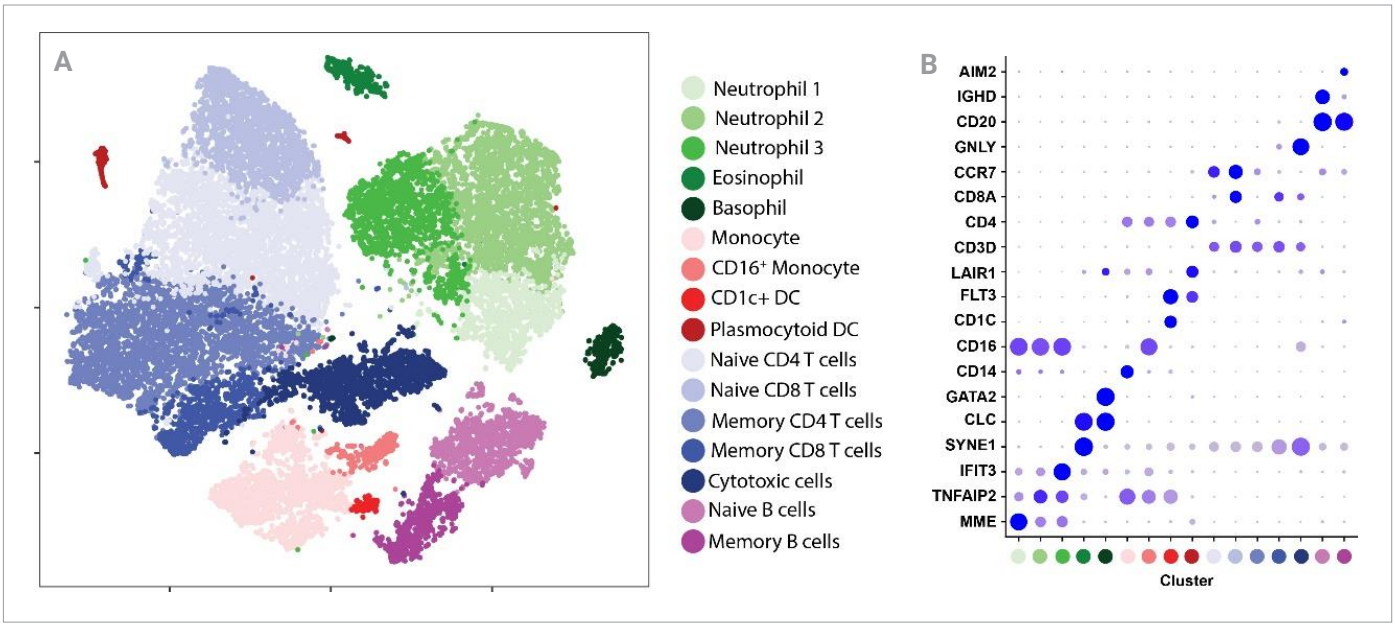

Figure 2A: TSNE plot showing high-quality single cells recovered from all six HIVE™ devices. Cells are colored by their cell-type identity.
Figure 2B: Dot plot showing the expression profile of canonical lineage markers (rows) for each cluster (columns)

When visualizing the cells colored by storage time point rather than cell-identity, the distribution is uniform within each cluster, indicating that cell-identity, rather than storage condition, is driving the clustering. Additionally, the relative frequency of each cluster is consistent between the two conditions. This is true even for granulocytes (green clusters in Figure 2A) that are challenging to recover after other storage methods such as cryopreservation.

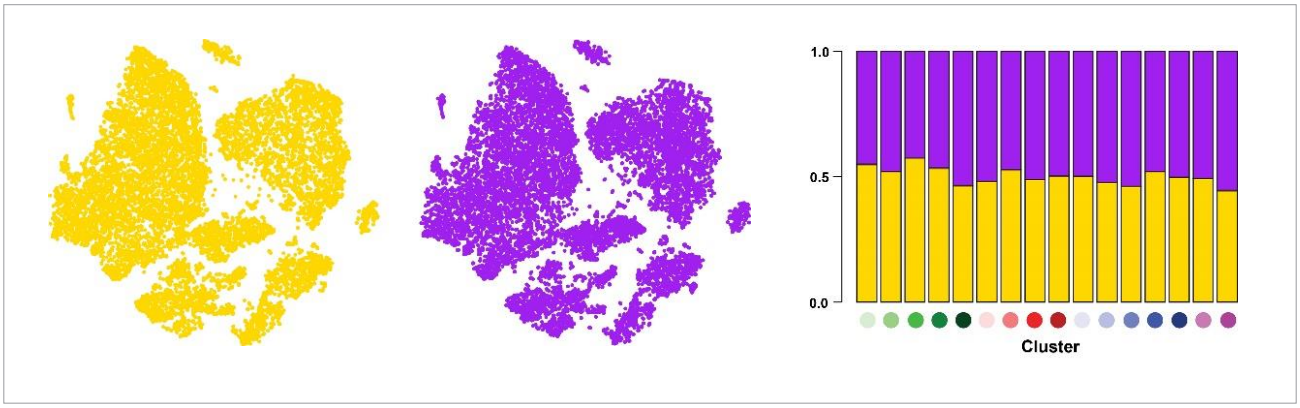

Figure 3A: TSNE plots for cells from HIVE™ devices processed fresh (day 0, gold) or after -20°C storage (day 14, purple).
Figure 3B: Relative frequencies, normalized to 1, for each cluster are maintained between the fresh (day 0, gold) and -20°C stored (day 14, purple) HIVE™ devices

There was no significant difference in cell number between the two storage conditions, with cell-loaded HIVE™ devices processed fresh (day 0) averaging 4,400 cells (SEM ± 302) and those stored at -20°C (day 14) averaging 3,800 cell (SEM ± 854). Median unique genes per cell and transcripts per cell were also consistent between the two conditions.

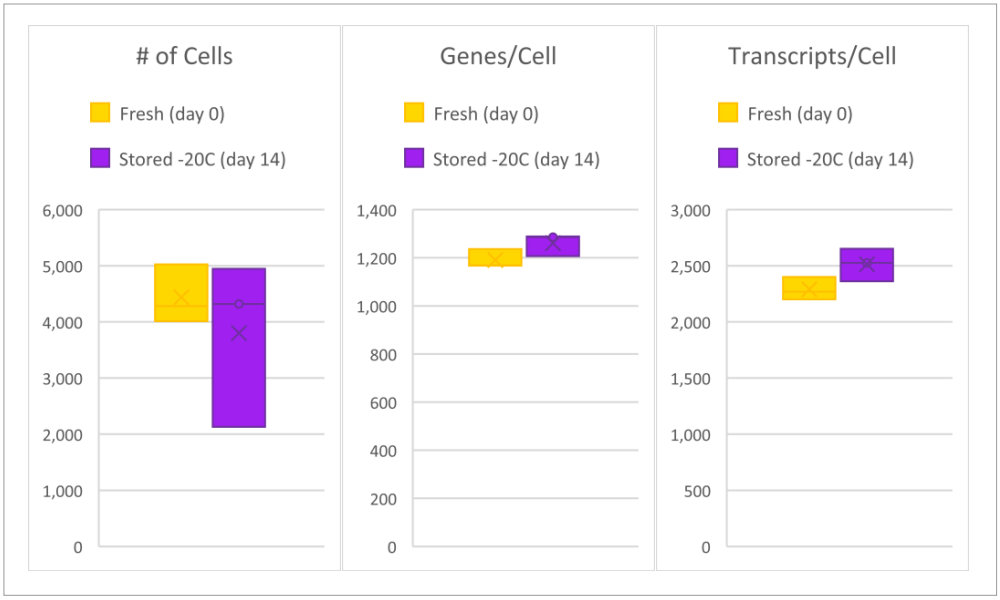

Figure 4: A) Number of high-quality single cells recovered. B) Median unique genes per cell. C) Median unique transcripts per cell. Gold = fresh (day 0), Purple = stored -20°C (day 14)

CONCLUSIONS

These results show that the quality and biological complexity of single-cell RNA-seq data generated using the HIVE™ scRNAseq Solution with integrated storage at -20˚C for 2 weeks are comparable to data generated from freshly processed samples.

Additionally, the HIVE™ scRNAseq solution can recover cells which are difficult to detect with other platforms because of their fragility. These include granulocytes, neutrophils, eosinophils, and basophils.

The HIVE™ device is a portable, handheld, single-use device that enables gentle capture, robust storage, and easy processing for the analysis of single-cell samples, solving the issues that limit single cell RNA analysis by:

- **Maintaining sample integrity throughout storage, shipping, and processing**
- **Enabling multi-site and multi-timepoint sample collection**
- **Increasing the recovery rates of fragile cells**

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