

## **Preventing Sample Bias in scRNAseq introduced by Cryopreservation: A Solid Tumor Case Study**

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**Objective:** Single cell RNA sequencing (scRNAseq) is sensitive to degradation during sample storage and shipping. Cryopreservation can cause poor cell viability due to ice crystal formation, leading to poor data quality. This creates a barrier limiting the ability of cores to offer scRNAseq services for users outside their facility. The HIVE™ scRNAseq Solution, developed by Honeycomb Biotechnologies, integrates stable single-cell storage and transcriptional profiling into a complete workflow enabling cores to overcome these limitations.

**Methods:** A fresh renal cell carcinoma biopsy was dissociated into a single-cell suspension for flow cytometry, cell capture with the HIVE™, and cryopreservation of the remaining sample. Cell-loaded HIVEs™ and cryovials were shipped to Honeycomb for processing and generation of HIVE™ libraries, after 2 or 20 weeks of storage. Libraries were sequenced on Illumina® NovaSeq® 6000 or NextSeq® 2000 platforms, and count matrix files were generated using BeeNet, Honeycomb's software specifically designed for HIVE™ libraries. Seurat was used for downstream analysis of high-quality cells.

**Results:** Cell-type proportions were matched between flow cytometry and HIVE™ scRNAseq Solution results, indicating no bias in cell-type recovery. Sequencing results were consistent across both Illumina® platforms. The sample stored in the HIVE™ was stable through 20 weeks and yielded 2–3× more cells with higher genes and transcripts per cell compared to the cryopreserved cells, which were immediately damaged after only 2 weeks of storage. The cryopreserved sample progressively degraded through 20 weeks of storage, specifically destroying immune cells, including T cells, monocytes, and macrophages, biasing sample composition.

**Conclusions:** The quantity, quality, and biological complexity of cells stored and shipped in the HIVE™ device are comparable to freshly processed cells. The HIVE™ scRNAseq Solution enables single-cell studies with remote sample collection and centralized lab processing, giving core facilities the ability to process samples collected across the globe.

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