LEVITAS BIO

Enabling superior performance with 10X Genomics Single Cell Sequencing

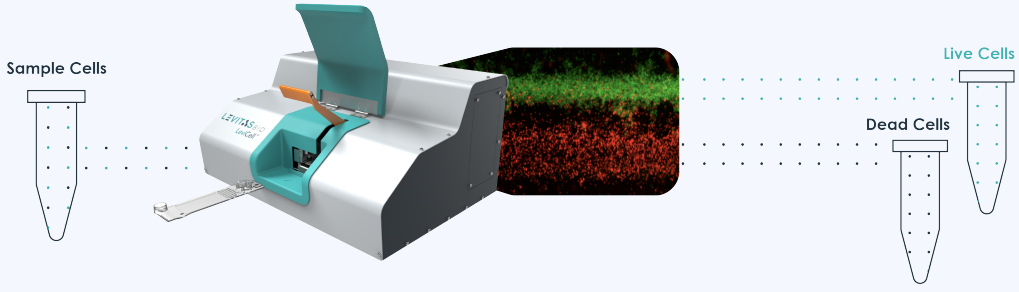
Introduction

The 10X single cell sequencing platforms have helped to revolutionize our understanding of biology and how the measurement of individual cells' gene expression can uncover the previously hidden messages commonly unseen in bulk mRNA experiments.

Unfortunately while the single cell sequencing workflow has proven to be fast, simple and reliable, obtaining the most complete and accurate single cell data depends on up-front sample preparation steps. Traditional methods currently in use tend to damage cells, have low capture efficiency, and often completely fail to process sensitive and difficult to work with samples such as dissociated tissue samples. The most common sample enrichment methods, Flow Cytometry and Bead-based enrichment methods, both often lead to significant cell stress and gene expression changes due to the high pressures used (Xiong et al., 2002; Romero-santacreu et al., 2009; Van Den Brink et al., 2017) or cell signaling effects due to the cell surface binding required by these methods. (Kornbluth and Hoover, 1989; Christaki et al., 2011). Due to these shortcomings many promising single cell experiments delivered biased results or fail to delivery results entirely.

The urgency for technological platforms that are gentle (to minimize cell damage), and efficient (to maximize the capture of all live cells in the population while maintaining the original cellular representation) is imperative to realize the scientific and clinical potential of single-cell analysis. The LeviCell demonstrates such a promise.

Methods



1 Sample Introduction
Sample pipetted into the LeviCell cartridge.

2 Automated Label-Free Sorting
Magnetic force drives levitation based on cells physical properties.

3 Sample Collection
Levitated cells transition into separate collection ports for removal.

LevitasBio has developed an innovative levitation technology platform which delivers a simple 3-step sample enrichment method. The LeviCell has been demonstrated to outperform standard processing in terms of cell viability, yield and most importantly the quality of single-cell results all in a fast and simple process that perfectly matched to any single cell workflow.

FACS		BEADS		LeviCell	
Total Time	116 min	Total Time	69 min	Total Time	21 min
Hands-on Time	46 min	Hands-on Time	34 min	Hands-on Time	2 min
Pipette Steps	9	Pipette Steps	11	Pipette Steps	2

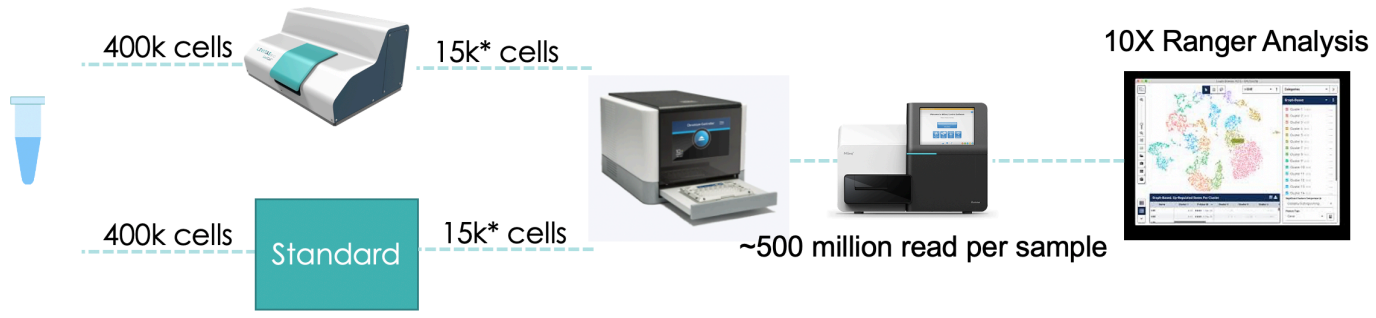
The simple three-step process streamlines the workflow and minimizes the risk of contamination and damage from multiple instances of handling and washing, consequently maximizing enrichment for each cell type. The LeviCell validated samples and cell types can be found on out website www.levitasbio.com and is continuously expanded.

Results

Two dissociated primary tumor samples, an early and late stage bladder tumor, were used for the analysis. Both were obtained from Discovery Life Sciences (DLS). The samples were dissociated following DLS protocols.

Library Preparation

To demonstrate the benefits of the LeviCell in removing dead cells and debris, each sample of 400k cells was split into fractions prior to the each enrichment process. One half of each sample was washed into a 10X library preparation buffer, while the other half was enriched for live cells using the LeviCell. After quantitation, the output from the LeviCell was put directly into the Chromium Next GEM single Cell 3' kit step without additional washing. Approximately 15k cells were used for each method. Final cDNA concentration was estimated by BioAnalyzer. While all samples succeeded at this step, there was a clear improvement in quality of the cDNA and the results library quality from the material generated by the LeviCell.

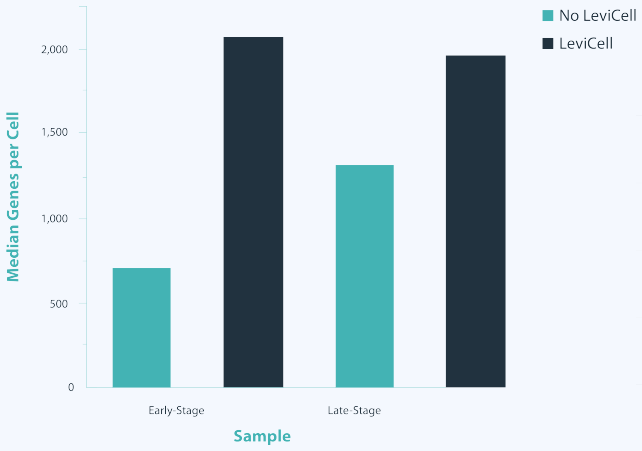


All samples were normalized before library preparation to account for differences yields, after normalization 15K cells were loaded from each process into the Chromium system.

Sequencing

All samples were sequenced on the Illumina 6000 S2 sequencer to a depth of ~500 million read per sample. Sequencing metrics, including Q30 bases in reads, barcodes, and UMI's were nearly identical across all samples. The fraction of reads mapped overall were very similar across all samples, and only small differences were observed in the fraction of reads mapping to intergenic, intronic, and exonic regions of the genome.

The most striking differences were seen when comparing the detected genes in the non-enriched sample to its corresponding live-enriched sample. This figure highlights the fact that the early stage bladder cancer had a nearly three-fold increase in the median number of genes detected per cell, while the late stage cancer sample showed a 50% increase.



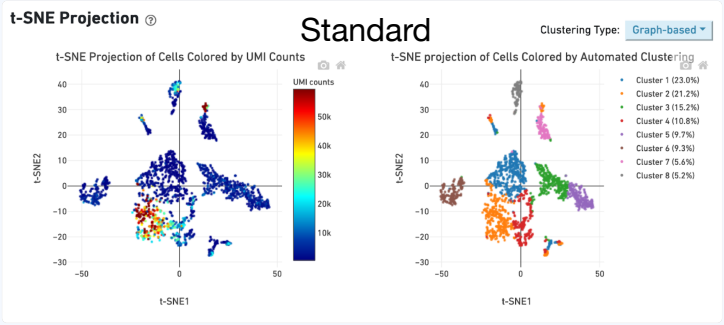
Sample	Method	Estimated Numer of Cells	Mean Reads per Cell	Median Genes per Cell	Number of Reads
Early-Stage	No LeviCell	1,890	279,155	697	527,603,273
Early-Stage	LeviCell	5,471	94,036	2,065	514,473,991
Late-Stage	No LeviCell	7,161	73,402	1,305	525,633,305
Late-Stage	LeviCell	8,058	67,266	1,951	542,036,469

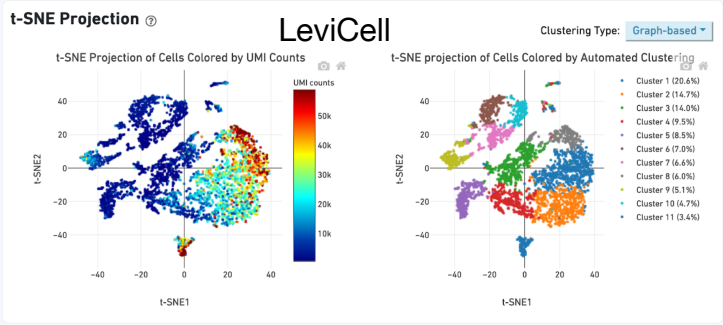
Sequencing Analysis

Sequencing results were analyzed using Cell Ranger from 10X Genomics. The plots below demonstrate the clear benefits of using the LeviCell to prepare samples for single cell sequencing. Samples enriched on the LeviCell produced a significantly larger number of genes detected per cell. This, combined with the better distribution of UMI counts, leads to a more complete picture for samples prepared using the LeviCell.

Early Stage Cancer Sample

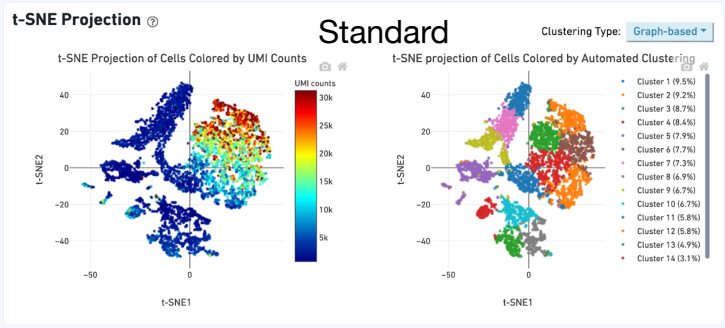
The distribution of UMI counts per cell in the standard method shows a small cluster of cells with the majority of the UMI counts. This same cluster of cells is represented by a single cluster when grouped by gene expression changes. In contrast, in the LeviCell enrichment sample there is a larger number of cells with significant numbers of UMI counts. This same group of cells is represented by four gene expression clusters (lower right), indicating a larger range of cellular diversity captured in the sequencing results.

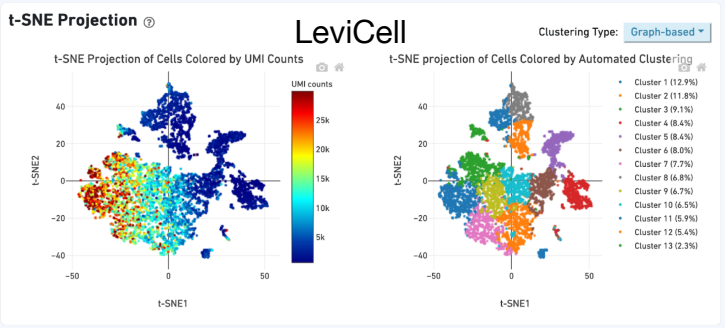




Late Stage Cancer Sample

A similar trend is observed with the late stage cancer sample, although the trends are less pronounced. Here, the sample processing with the standard method shows a large number of cells with high diversity of UMI counts, and correspondingly five clusters of gene expression. The sample after LeviCell enrichment has a larger number of cells with high diversity of UMI counts, and six clusters of gene expression clusters, indicating a superior view of the expression profiles.





Conclusion

The innovative label-free separation technology of the LeviCell facilitates complete debris and dead cell removal without affecting the original population representation of gene expression (please see our Population Representation application note for additional data on how the LeviCell maintains the original population representation). When it comes to single-cell study, purity and viability of cells harvested from the sample preparation stage is a determining factor for generating high quality data in downstream assays such as NGS (Next Generation Sequencing). The LeviCell's ability to seamlessly enrich for target cells and produce robust yields of viable cells without preferentially depleting or changing the frequency or expression of cell types gives it the necessary technological characteristics that many scientists have been waiting for to take them to the next level.

1455 Adams Drive
Menlo Park, CA 94025
contact@levitasbio.com