

Top tips for elevating your single-cell sequencing experiments.

Single-cell sequencing is a rapidly evolving approach to delve into the genomic, transcriptomic, epigenomic, and proteomic landscapes of single cells. Compared to traditional bulk sequencing methods, which often fail to capture the rich variability present among individual cells, single-cell sequencing offers the opportunity to investigate cellular heterogeneity across a wide range of tissues and cell populations.

By providing fresh insights into the complexity of cellular populations, single-cell sequencing can elucidate rare subpopulations, dynamic cellular states, and the molecular determinants underlying cellular diversity, developmental processes, and disease pathogenesis.

Over the past decade, significant advancements in technology have facilitated the widespread adoption of single-cell sequencing methodologies. These technological innovations have not only increased the throughput and sensitivity of single-cell analysis but have also significantly reduced the cost and complexity associated with experimental procedures.

By delving into the molecular intricacies of single cells, researchers can uncover predictive biomarkers and therapeutic targets with clinical implications for a wide range of diseases. However, despite these remarkable strides, challenges remain in the analysis and interpretation of single-cell data.

In this article, we explore some of the prevalent challenges within single-cell studies and discuss emerging strategies to overcome them.



Four key challenges associated with single-cell studies include:

1. Analytical burden and inefficient data interpretation

Streamlining the data analysis process is vital to conserve valuable resources and enhance accurate data extraction.

2. Low sample viability

Enhancing sample viability is paramount to improve the robustness and reproducibility of downstream analyses.

3. RNA dropout

Overcoming RNA dropouts is crucial to enhance the accuracy of single-cell analysis.

4. Inaccurate nuclei counting

Ensuring accurate nuclei counting is essential for reliable downstream analyses.

1. Preventing analytical burden and inefficient data interpretation

The challenge

Single-cell multi-omics experiments generate large amounts of high-dimensional data that require advanced tools and expertise to process and interrogate. Even with highly skilled bioinformaticians available, defining relevant and biologically meaningful cell populations for subsequent analyses remains challenging due to the complexity inherent to the data. This can lead to unnecessary rounds of analysis, consuming precious time and resources; ultimately delaying the answers investigators require.

TOP TIP

Empower researchers with preliminary analysis

Empowering researchers to independently identify cell populations of interest before involving bioinformatics experts for detailed analysis can streamline the process and avoid unnecessary rounds of investigation.

“Multi-omics Analysis Software is not designed to replace the bioinformaticist; rather, it is designed to supplement and supercharge the ability of the bioinformaticist and biologist to interface, accelerating time to results.”

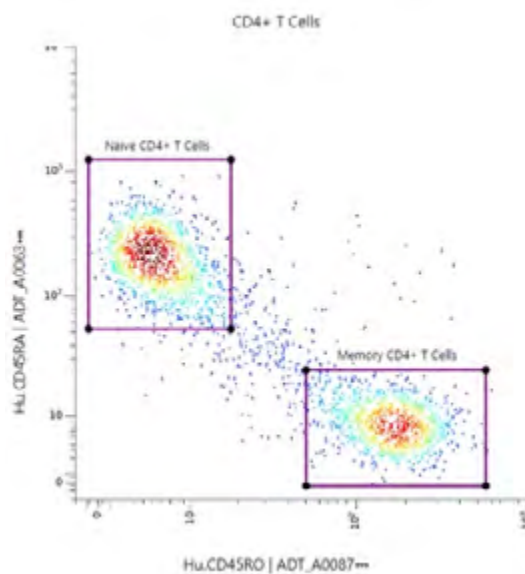
Josh Croteau, Program Manager,
Multi-omics, BioLegend



TECHNOLOGY SOLUTION

BioLegend's Multi-omics Analysis Software

BioLegend's cloud-based Multi-omics Analysis Software (MAS) serves as an accessible software solution to streamline single-cell multi-omics analysis. The software's user-friendly interface enables biologists to independently explore, iterate, and draw conclusions from their data, putting scientists back in the driver's seat of their analyses. MAS equips scientists with the tools to confidently perform various downstream assessments, including normalization, cell annotation (gating or clustering), multiplet removal, and differential protein and gene expression investigations. Whether researchers have robust bioinformatics support or are operating autonomously, MAS emerges as an indispensable tool for gaining a head start in single-cell analysis and prevent unnecessary rounds of investigation.



2. Ensuring high sample viability for sensitive and meaningful data

The challenge

Ensuring high sample cell viability is a critical factor in the success of single-cell workflows. Many single-cell sequencing platforms recommend a minimum viability threshold of 80% or above to ensure that the generated data is a true representation of the cell population. Low cell viability jeopardizes data relevance due to sample degradation and wastes resources by committing sequencing to low-quality samples. Ultimately, this compromises the utility of single-cell work. Meticulous attention to sample handling and viability optimization is therefore essential to overcome this challenge and improve the utility of single-cell sequencing experiments.

TOP TIP

Use of dead cell removal kits for improved viability

Dead cell removal kits can be used to selectively remove dead and dying cells from a cell population. By employing negative selection of live cells, the impact of low cell viability can be mitigated before samples are loaded into a single-cell workflow.

“One of the fundamental challenges in single-cell sequencing lies in the viability and quality of the samples entering the workflow. Our dead cell removal kits serve as a proactive solution to mitigate the presence of dead or dying cells prior to loading samples into a single-cell workflow.”

Ashley Cornett, Senior Manager,
Scientific Applications, BioLegend

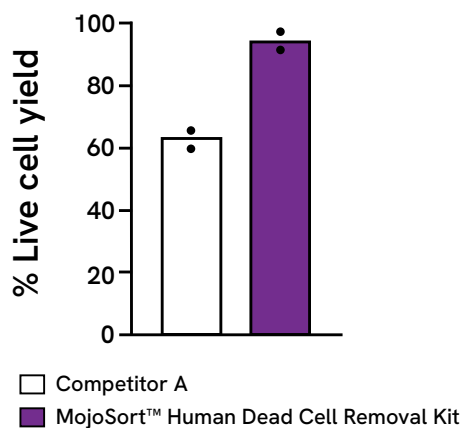


TECHNOLOGY SOLUTION

BioLegend's magnetic cell separation system MojoSort

BioLegend's MojoSort™ serves as a technological solution to enhance sample viability in single-cell workflows. The magnetic cell separation system can be used to remove dead and dying cells, addressing the challenge of low viability. MojoSort also provides researchers with the flexibility to employ positive or negative selection strategies, allowing for the additional removal of unwanted cells and/or enrichment of cells of interest from heterogeneous populations.

This dual functionality not only improves viability but also offers the flexibility to isolate and purify specific cell subsets for single-cell workflows, contributing to more accurate and reliable data generation.



3. Reducing the impact of RNA dropout

The challenge

Single-cell RNA-sequencing (scRNA-seq) offers insights into cellular heterogeneity and function by quantifying gene expression at the individual cell level. However, scRNA-seq faces a challenge known as 'RNA dropout' where lowly expressed genes with limited transcript numbers in the cell are missed by the chosen RNAseq approach. These RNA dropouts hinder the analysis, resulting in an incomplete representation of the transcriptome for a given cell, impacting data quality.

TOP TIP

Implement CITE-seq for comprehensive profiling

Incorporating protein detection to experimental strategies to overcome the challenge of RNA dropouts and enhance the accuracy of analyses. One useful approach is CITE-seq (Cellular Indexing of Transcriptomes and Epitopes by Sequencing), which combines gene expression profiling with the measurement of protein markers on individual cells. This dual-modality profiling enables a comprehensive understanding of both the transcriptomic and proteomic profiles of individual cells simultaneously.

"Part of the challenge with RNA dropout is that a biologically relevant marker within a specific cell type may go undetected, not because the transcript isn't being made, but rather due to the lower abundance of RNA meaning it is not captured in downstream analysis pipelines. In contrast, protein is more abundant which allows for more consistent detection and aides in further characterization of subsets of cell types."

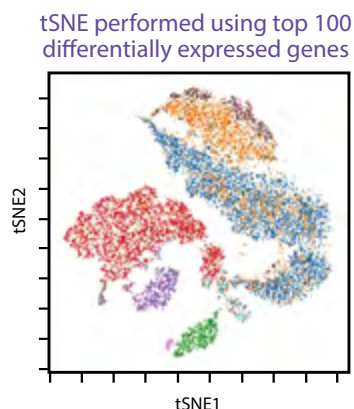
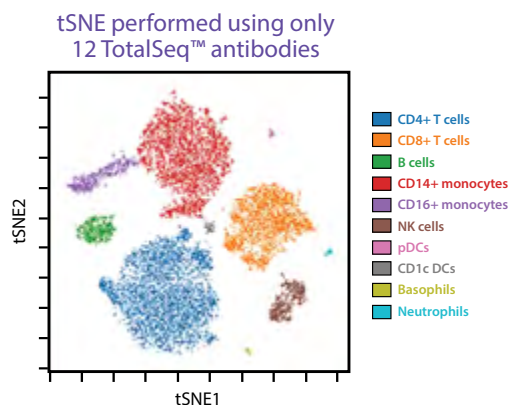
Ashley Cornett, Senior Manager,
Scientific Applications, BioLegend



TECHNOLOGY SOLUTION

BioLegend's TotalSeq reagents

The incorporation of oligonucleotide-labeled antibodies, such as BioLegend's TotalSeq™ reagents, into workflows like CITE-seq serve as a technological solution to reduce an experiments vulnerability to RNA dropout by adding protein information to help identify cells and understand their biology. TotalSeq reagents provide a method for measuring protein and RNA simultaneously in single cells. These antibody-oligonucleotide conjugates carry unique barcodes that can be associated with RNA transcripts derived from the same cell during the sequencing process. By integrating information about gene expression and proteins at the single-cell level, CITE-seq enables a more detailed characterization of cellular heterogeneity and function, enhancing the accuracy and reliability of scRNA-seq analyses.



4. Increasing accurate nuclei counting for enhanced sample quality

The challenge

Accurate nuclei counting is crucial in single-cell workflows, particularly during library preparation, to minimize unusable cells and ensure debris-free samples. In addition, downstream analysis requires reliable data on cell population heterogeneity which can be influenced by poor sample preparation due to low viability and large aggregates. Automated cell counting provides a reliable and consistent method to detect isolated cells/nuclei where manual counting can be challenging due to their small size, particularly when dealing with a large number of cells.

TOP TIP

Embrace automated technologies for nuclei counting

Visualization and enumeration of individual cell nuclei can be achieved using fluorescent dyes or markers that specifically bind to DNA. The inherent difficulty in manual counting, especially in the presence of cellular debris, may necessitate the adoption of automated cell counting techniques to enhance accuracy.

“One of the greatest challenges is ensuring samples meet quality criteria. If a sample isn’t clean, then why would you submit it for sequencing? This is where our instrumentation is wonderful – it can give scientists valuable information prior to submitting a sample, which can save them a lot of time and money.”

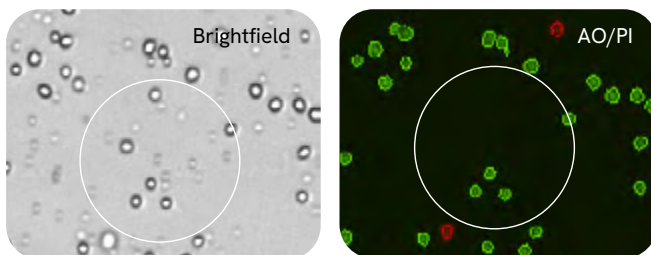
Charles Hernandez,
Field Application Scientist
Manager, Revvity



TECHNOLOGY SOLUTION

Cellaca PLX high-throughput cell and nuclei counter

The use of automated cell counters, such as the Cellaca™ PLX high-throughput cell and nuclei counter, emerges as a technological solution to improve nuclei counting accuracy in single-cell workflows. Automation not only handles large sample quantity efficiently but also removes user bias from the cell counting process. By adopting automated methods, researchers enhance the accuracy and reliability of nuclei counts compared to manual counting approaches. Moreover, automated counters enable the adoption of standardized methods, improving reproducibility and streamlining workflows. The Cellaca PLX also boasts a relatively low volume requirement. This feature is particularly beneficial when sample material is limited, allowing users to preserve the majority of the sample for their intended experimental purposes.



Dual fluorescent imaging enables detection of viable nuclei

[Click here](#) for more information about single-cell sequencing.



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