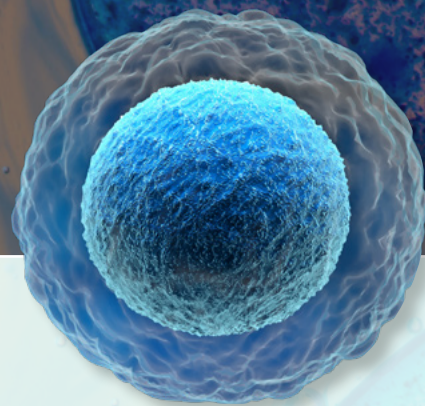


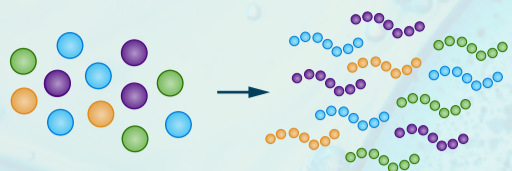
# Tips for a Successful Single-Cell Experiment



By measuring levels of gene expression, RNA-seq provides insights into how cells and tissues function.

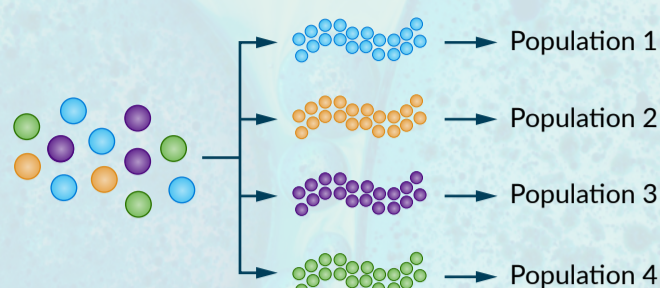
## Bulk RNA-seq

Bulk RNA sequencing data represents an average of gene expression across a particular tissue system; it is most useful for comparative transcriptomics as well as determining disease biomarkers.



## scRNA-seq

In contrast, scRNA-seq provides a detailed analysis of the transcriptome for individual cells by isolating and barcoding them before sequencing. This single-cell expression data is useful for studying heterogeneity within a tissue and the variability of gene expression in a particular cell population.



## scRNA-seq Methods and Characteristics

scRNA-seq methods typically fall into one of four distinct groups: Nano/microwell-based, cell sorting-based, droplet/microfluidics-based, or low-equipment technologies.

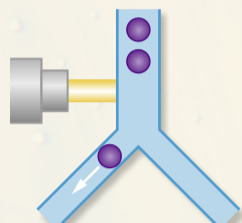
### Nano/microwell-based methods

Including Seq-Well, the HIVE, and BD Rhapsody, employ miniaturized wells to separate individual cells for analysis with high scalability.



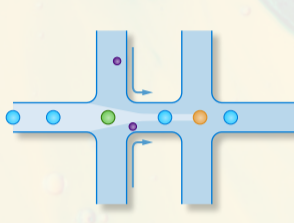
### Cell sorting-based methods

Including SCRB-seq and Smart-seq2, utilize technologies such as fluorescence-activated cell sorting for precise cell selection and isolation.



### Droplet/microfluidics-based methods

Including Chromium, inDrop, and Drop-seq, rely on droplets and microfluidic technologies to isolate cells and deliver reagents for high-throughput analysis.



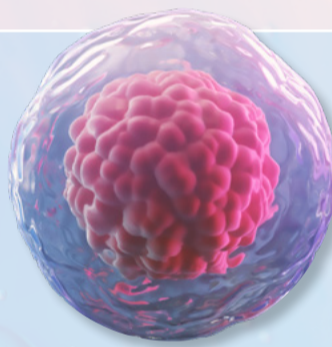
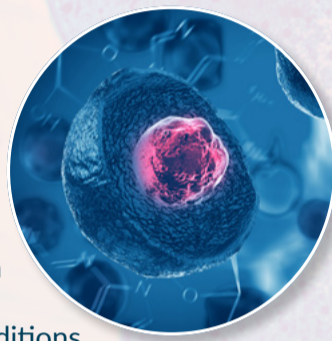
### Low-equipment methods

Including PIPseq, SPLiT-Seq, and sci-RNA-seq, only require common lab equipment and involve simplified workflows for single-cell studies.



## Deciding which methods to use depends on several key factors

- **Throughput:** High-throughput methods can process more cells simultaneously. While ideal for large-scale studies, this may reduce the resolution needed for tasks such as identifying low-level transcripts.
- **Costs:** Many advanced and high-throughput methods have higher costs. Budget constraints may require more cost-effective options that have limitations in other areas.
- **Technical and instrument requirements:** Some methods require specialized instruments and technical expertise. Simpler and less resource-intensive approaches are more accessible for labs with basic setups.
- **Sample type:** Cell types and their condition can dictate compatibility. Certain methods have limitations on the cell types, sizes, and conditions they can handle.
- **Gene coverage:** Each library prep associated with a technology differs in gene coverage capabilities (e.g., 5', 3', or full-length). The gene coverage needed for proper analysis can influence the method selection.



## Best Practices to Obtain a High-Quality Sample

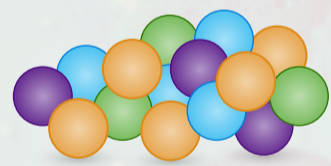
Regardless of the scRNA-seq platform used, a successful sample prep is essential for producing high-quality data. Samples should have high viability (more than 90% viability is ideal), and intact cell membranes. A good single-cell suspension with no clumps is also important, as well as a clean prep with little or no debris. Below are seven tips to help you prepare a high-quality sample.

- 1 Choose a dissociation protocol optimized to your cell type of interest
- 2 Remove dead cells and adjust to the required cell density
- 3 Perform a pilot experiment
- 4 Reduce the time spent during sample prep
- 5 Maintain samples at a low temperature
- 6 Be gentle during sample manipulation
- 7 Consider single nucleus sequencing for difficult samples

*Ideal lysis and isolation conditions include low temperatures and short timeframes. Pipetting should be gentle as well as any centrifugation (300–500 xg). Some protocols benefit from using BSA (up to 1%) or FBS (up to 2%). Debris should be removed by filter or density medium.*

## How Many Cells Need to Be Analyzed and How Deep Should the Sequencing Be?

There is no easy answer to this question and the best response would be: It depends on the question. However, here are four recommendations that shed some light on best practices.



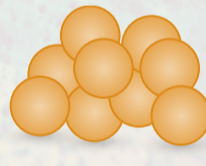
### diverse tissue = more cells

For tissues or samples containing many cell types, a larger number of cells may need to be analyzed to capture the full range and diversity.



### rare cell type = more analysis

With a rare cell type of interest, you need to analyze many cells.



### heterogeneity = fewer cells

For overall heterogeneity, fewer cells might be enough.



### rare transcript = increased sequencing depth

The rarer the transcript, the higher the sequencing depth should be.

## Batch Effects in scRNA-seq: What Causes Them and How to Remove

Data integration and interpretation can be impacted by batch effects, which have many sources including differences in sample handling, library preps, batches of reagents, sequencing runs, and operator variability. To avoid this common problem:



Use good experimental design



Maintain the same operator to minimize handling variation



Include biological replicates



Keep equipment calibrated and uniform for the experiment



Collect and process samples at the same time



Prepare and sequence libraries together



Utilize a consistent batch of reagents throughout the experiment



Choose computational tools that remove variation from analysis

## References

- 1 <https://www.10xgenomics.com/blog/sample-preparation-tips-for-your-single-cell-rna-seq-experiments>
- 2 <https://www.biocompare.com/Editorial-Articles/599223-Factors-to-Consider-When-Selecting-an-scRNA-seq-Method/#:~:text=Key%20Considerations%20for%20Method%20Choice,when%20selecting%20scRNA%2Dseq%20methods.>
- 3 <https://www.biocompare.com/Editorial-Articles/597603-Single-Cell-Sequencing-Best-Practices-and-Advice-for-Sample-Prep/>
- 4 <https://www.takarabio.com/about/bioview-blog/tips-and-troubleshooting/5-tips-to-make-your-single-cell-rna-seq-experiments-a-success>
- 5 <https://www.biocompare.com/Editorial-Articles/596292-Optimizing-Sample-and-Library-Prep-for-Single-Cell-RNA-Seq/>
- 6 <https://www.10xgenomics.com/resources/analysis-guides/introduction-batch-effect-correction>