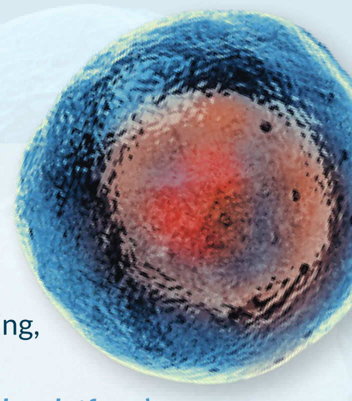
Single-Cell Sequencing Steps and Methods

Single-Cell Sequencing Steps



Isolation

Common methods for single-cell isolation include using microfluidic devices, fluorescence-activated cell sorting (FACS), magnetic-activated cell sorting (MACS), laser capture microdissection (LCM), manual manipulations, and combinatorial barcoding, which doesn't require complete separation.

! *Before selecting an isolation method, evaluate your project to ensure it aligns with the platform's criteria, including cell viability, library compatibility, throughput, and costs.*

Library Preparations

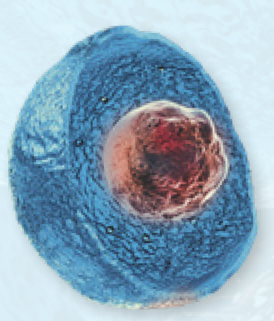
The library preparation step converts the target DNA or RNA into sequencing-ready libraries that are pooled (i.e., combined) and sequenced together. Quality control is a crucial part of this process to ensure an accurate representation of individual genomes, transcriptomes, or epigenomes.

! *Single-cell library preps vary greatly depending on the target of interest, and many isolation methods are directly integrated into this process.*

Sequencing

The expansion of single-cell sequencing allows researchers to employ various commercial sequencers for their studies. The key consideration is the compatibility with specific library preparation methods and determining if the research goals are better suited for long-read or short-read platforms.

! *Single-cell studies often require a high number of reads to accurately capture heterogeneity. Choose a setup that exceeds the required depth and coverage to account for any variations.*



Data Analysis

While data analysis is typically the final stage, deciding on the analytical method and software is crucial before launching a single-cell study. The datasets produced are large and complex and may require specific computational infrastructure and analysis expertise.

! *Open-source options are free and typically flexible, but they require coding and analysis knowledge. Commercial options are easier and don't require any expertise, but they are not free and not always customizable.*

Sequencing Methods

Single-Cell Genomics

Researchers use scDNA-seq to investigate genomic variations (e.g., SNVs, CNVs, SVs), chromosomal aneuploidy, lineage tracing, genome integrity, cellular differentiation, and tumor heterogeneity. The research goal determines whether targeted sequencing or whole genome sequencing is required.

! *Many amplification strategies exist for scDNA-seq, which differ based on coverage, accuracy, and uniformity.*

Single-Cell Transcriptomics

Applications of scRNA-seq encompass the whole transcriptome, 3′ mRNA ends, full-length transcripts, non-poly(A) RNAs, specific gene targets, and spatial expression patterns. The choice of preparation method depends on the resolution, RNA type, and research question.

! *Cell preparations can alter transcription, affecting results. snRNA-seq is a suitable alternative for various tissues.*

Single-Cell Epigenomics

Single-cell epigenomic sequencing includes methods for studying chromatin accessibility (scATAC-seq), protein-DNA interactions (scChIP-seq), methylation patterns (scBS-seq, scWGBS, scRRBS), chromatin interactions (scHi-C), and multi-omic approaches (sn-m3C-seq).

! *Combining epigenomic techniques with scRNA-seq is common to evaluate its effect on expression within the cell.*

Single-Cell Proteomics

The standard approach involves isolating cells and their proteins, followed by identification through mass spectrometry. A significant hurdle in single-cell proteomics is the inability to amplify proteins like is done with nucleic acids.

! *Single-cell proteomic techniques using NGS are indirect and measure protein abundance through barcode-labeled antibodies (e.g., CITE-seq and REAP-seq).*

Common Challenges and Solutions

	Challenge	Solution
1	Introduced bias can occur from the tissue and cell isolations, as well as the library prep	Optimize dissociation protocols, use spike-in controls, and unique molecular identifiers (UMIs)
2	Failed capture or amplification of targets (dropout) can be misinterpreted as a false negative	Choose improved amplification methods and computational approaches that impute missing data
3	Low-quality samples and libraries can introduce noise and cause misleading results	Perform QC on libraries before sequencing and use validated data normalization methods
4	Data integration, interpretation, and in-depth analysis are particularly difficult for these datasets	Carefully select established analysis workflows and consult bioinformatics experts