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Single-Cell RNA-Seq Workflow Guide

A practical map from cell-by-gene counts to QC, clustering, markers and cell-type interpretation.

Workflow

Step	Purpose	Typical outputs
1. Experimental design	Define biological groups, replicates and capture strategy.	Sample metadata
2. Generate count matrix	Process raw reads to cell-by-gene counts.	Matrix + barcodes + features
3. Cell QC	Filter low-quality cells, doublets and problematic features.	QC-filtered matrix
4. Normalization	Account for library-size and technical effects.	Normalized values
5. Feature selection	Identify informative genes.	Variable features
6. Dimensionality reduction	Represent major variation.	PCA / UMAP
7. Clustering	Group transcriptionally similar cells.	Cell clusters
8. Marker analysis	Identify genes characteristic of clusters.	Marker table
9. Cell annotation	Assign biological identities using evidence.	Cell labels
10. Comparative analysis	Compare cell states or proportions across conditions.	Differential results

Avoid over-annotation: Cell-type labels should be supported by multiple markers and biological context. QC thresholds are dataset-specific, not universal constants.