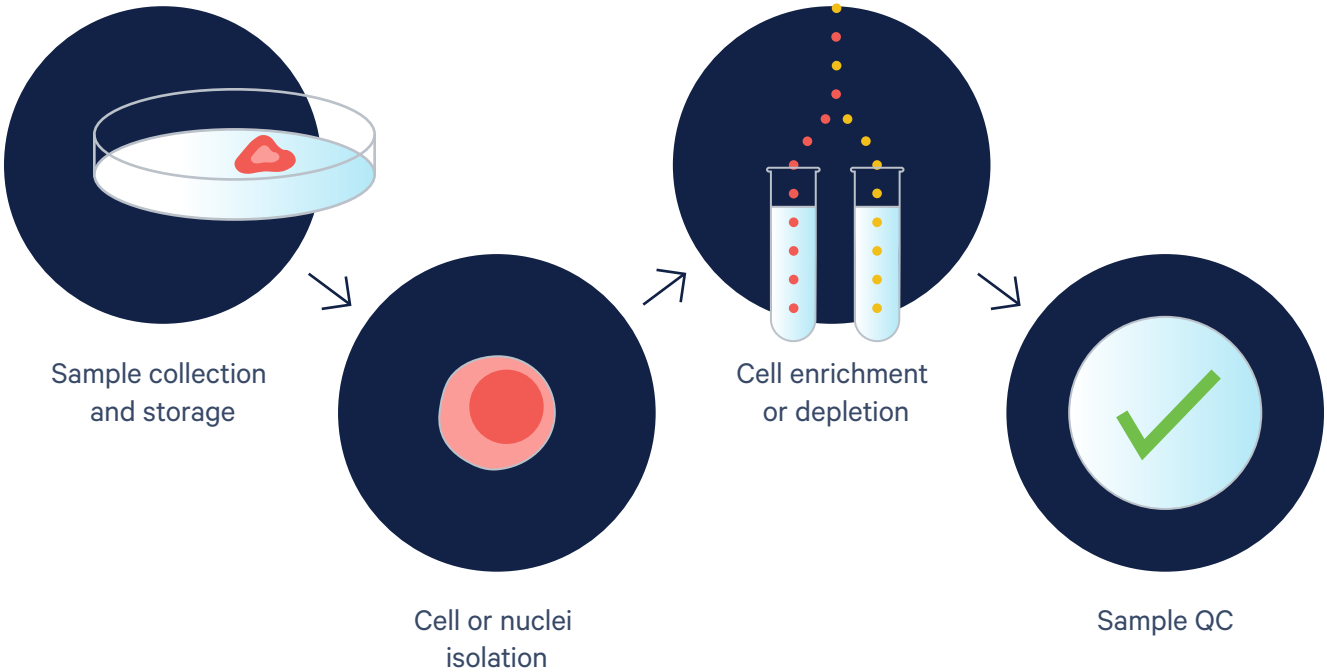
10x GENOMICS

Sample preparation optimization

Single Cell Gene Expression LT



Why use the Single Cell Gene Expression LT kit for optimizing sample preparation?

Before embarking on a full-scale single cell sequencing experiment, it's best to determine the optimal sample preparation workflow, particularly for unique sample types. A variety of factors can be tested, including fresh versus frozen samples, mechanical versus enzymatic dissociation, optimal lysis time for nuclei isolation, and whether cells need to undergo additional enrichment or cleanup steps. The Single Cell Gene Expression LT kit lets you test all these variables with clear, sequencing-based readouts analogous to our standard Single Cell Gene Expression kit, but at lower cost and with fewer cells. In addition, the workflow is comparable to our standard gene expression kits, letting you practice all the key steps involved in single cell analysis.

Use case #1: Tissue dissociation methods

Numerous tissue dissociation methods are available, each with its own advantages and disadvantages. For fresh tissue, many researchers use enzymatic dissociation, which can provide high cell yield and viability. There are many different enzymes available for dissociation, and resources exist to match the best enzyme to your tissue and organism of choice. Keep in mind that some enzyme treatments may modify cell surface proteins and therefore would not be compatible with Feature Barcode technology for Cell Surface Protein, flow cytometry, or other orthogonal assays. Enzymatic dissociation can also be time consuming. An alternative is mechanical dissociation, which reduces processing time and works well with easy-to-dissociate sample types. However, cell recovery efficiency and cell viability can vary across users and experiments.

Recently, researchers have identified differences in cell-type composition and gene expression between enzymatic and mechanical dissociation for certain sample types. O'Flanagan et al. (2019) found that using enzymatic dissociation at cold temperatures helped minimize stress responses during mouse tumor dissociation. Mattei et al. (2020) observed differences in transcriptional profiles of cells isolated from brain tissue using enzymatic dissociation at 37°C or mechanical dissociation at 4°C.

When deciding whether enzymatic or mechanical dissociation is best suited for your experimental goals, consider assessing the differences in gene expression, cell surface protein expression, and cell-type distribution by performing a side-by-side comparison of each method in a low-throughput experiment using Chromium Single Cell Gene Expression LT. An informed decision can then be made based on empirical data before proceeding with additional samples.

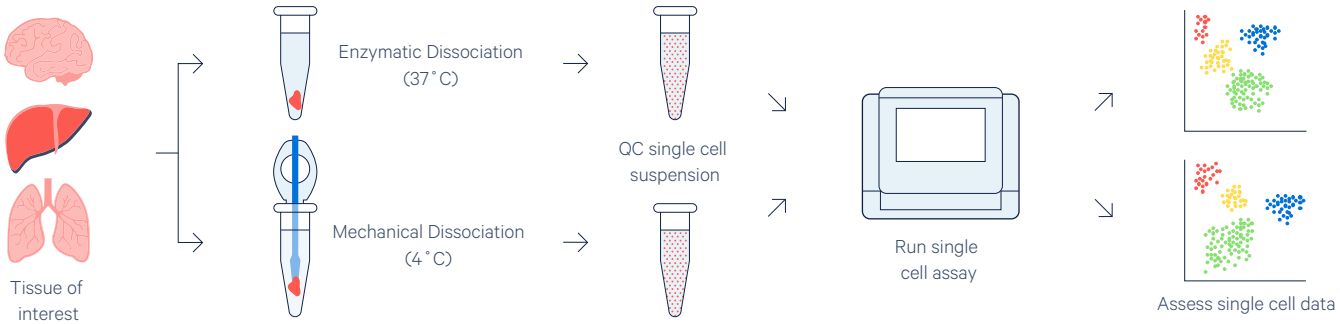


Figure 1. Recommended workflow for testing tissue dissociation methods. For each tissue of interest, prepare single cell suspensions side-by-side using enzymatic or mechanical dissociation, along with any other method variables of interest. Use Single Cell Gene Expression LT to compare cell composition and data quality of a small number of cells for each preparation method.

Use case #2: Nuclei isolation and cleanup methods

When working with frozen or difficult-to-dissociate tissue, nuclei isolation may be the best choice. Cell-type populations recovered from single cell or single nuclei RNA-seq have been found to be comparable in both published literature (Habib et al. 2017, Bakken et al. 2018, Korrapati et al. 2019, Pimpalwar et al. 2020) and our own in-house observations. For some considerations, nuclei may work better than cells for RNA sequencing, including reducing biases due to cell dissociation, minimizing stress-induced transcriptional artifacts, reducing microfluidic cell capture bias based on cell size, and capturing nascent mRNA for temporal studies.

However, cellular debris is generated during nuclei isolation as tissue is dissociated and cells are lysed. When the level of debris is high, consider incorporating a sample cleanup step. The cleanup method chosen can impact nuclei yield, quality, and total hands-on time. Some cleanup methods separate debris based on size or density, which may not be appropriate for complex samples with varying cell sizes. Access to instrumentation, such as cell sorters, and researcher expertise can also influence which methods are preferred.

Single Cell Gene Expression LT can be used to assess different cleanup methods before beginning a large-scale experiment. See the table below for a partial list of methods that can be used.

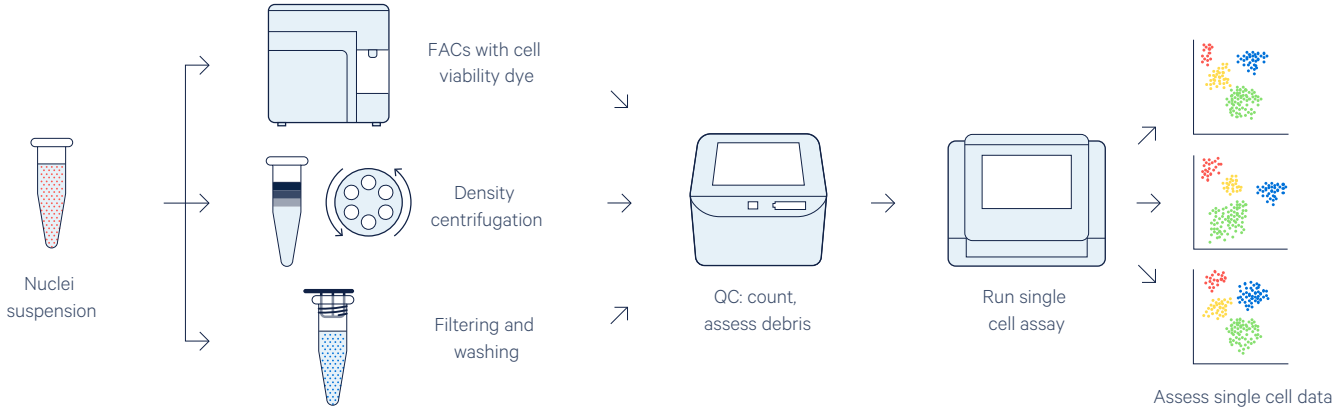


Figure 2. Recommended workflow for testing nuclei cleanup methods. When evaluating which cleanup method is best for your workflow, recovery of only a few nuclei is required. Single Cell Gene Expression LT lets you determine the level of background signal using the web summary, fraction of reads in cells, and overall cell clustering performance. Compare the impact of different cleanup methods on nuclei yield, cell debris, and ambient RNA to determine which cleanup method is best for future experiments.

Cleanup methods comparison

	FACS	Magnetic beads	Density centrifugation	Filtering	Washing
Debris removal	Yes	Yes	Yes	Yes	Yes
Low input samples	No	No	No	Yes	Yes
Nuclei	Yes	Maybe	Yes	Yes	Yes

References

1. Bakken TE, et al. Single-nucleus and single-cell transcriptomes compared in matched cortical cell types. *PLoS One* 13: e0209648, 2018.
2. Chongtham MC, et al. Isolation of nuclei and downstream processing of cell-type-specific nuclei from micro-dissected mouse brain regions – techniques and caveats. *bioRxiv*, 2020. doi: 10.1101/2020.11.18.374223
3. Habib N, et al. Massively parallel single-nucleus RNA-seq with DroNc-seq. *Nat Methods* 14: 955–958, 2017.
4. Heng BC, Cowan CM & Basu S. Comparison of enzymatic and non-enzymatic means of dissociating adherent monolayers of mesenchymal stem cells. *Biol Proced Online* 11: 161–169, 2009.
5. Ho H, et al. A guide to single-cell transcriptomics in adult rodent brain: The medium spiny neuron transcriptome revisited. *Front Cell Neurosci* 12: 159, 2018.
6. Korrapati S, et al. Single cell and single nucleus RNA-seq reveal cellular heterogeneity and homeostatic regulatory networks in adult mouse stria vascularis. *Front Mol Neurosci* 12: 316, 2019.
7. Mattei D, et al. Enzymatic dissociation induces transcriptional and proteotype bias in brain cell populations. *Int J Mol Sci* 21: 7944, 2020.
8. O'Flanagan CH, et al. Dissociation of solid tumor tissues with cold active protease for single-cell RNA-seq minimizes conserved collagenase-associated stress responses. *Genome Biol* 20: 210, 2019.
9. Pimpalwar N, et al. Methods for isolation and transcriptional profiling of individual cells from the human heart. *Heliyon* 6: e05810, 2020.
10. Worthington Tissue Dissociation Guide

Resources

Sample collection and storage

- Chromium Single Cell Applications - Guidelines for Optimal Sample Preparation - Technical Note (CG000126 Rev A)
- How can I ship cells?
- How can I ship tissue for 3' Gene Expression profiling?

Cell or nuclei isolation

- How can I isolate nuclei for 3' Gene Expression profiling?
- How do I dissociate my tissue of interest?
- What are the best practices for working with nuclei samples for 3' single-cell gene expression?

Cell enrichment or depletion

- Enrichment of CD3+ T Cells from Dissociated Tissues for Single Cell RNA Sequencing and Immune Repertoire Profiling - Demonstrated Protocol (CG000123 Rev B)
- Removal of Dead Cells from Single Cell Suspensions for Single Cell RNA Sequencing - Demonstrated Protocol (CG000093 Rev C)
- What are the best practices for flow sorting cells for 10x Genomics assays?

Sample QC

- Chromium Single Cell Applications - Guidelines for Optimal Sample Preparation - Technical Note (CG000126 Rev A)
- Guidelines for Accurate Target Cell Counts Using 10x Genomics® Single Cell Solutions - Technical Note (CG000091 Rev B)
- Interpreting Cell Ranger Web Summary Files for Single Cell Gene Expression Assays -Technical Note (CG000329 Rev A)

Contact us

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