

## **To multiplex, or not to multiplex**

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Single-cell sequencing is now a mainstay of genomics research, and although there are a multitude of experimental designs to match a wide array of research questions, optimal strategies for multiplexing remain unclear. Sample multiplexing involves pooling multiple samples together, sequencing them, and subsequently deconvoluting them computationally. The high-throughput of modern single-cell technologies makes multiplexing realistic for many applications.

Deciding whether to multiplex samples is a key consideration in the planning of single-cell experiments. We focus on publicly available transcriptomics data from the 10X Genomics platform to explore how best to apply sample multiplexing. We expand on the consequences of choosing whether or not to multiplex on downstream analysis, and provide recommendations for researchers depending on key experimental factors. In particular, this choice impacts important steps in single-cell workflows including duplicate detection and batch effect correction. There is a trade-off between the minimisation of batch effects that sample pooling provides, and the reduced throughput of sequencing samples together, as opposed to creating separate libraries. If choosing to proceed with a multiplexed approach, researchers are also faced with a decision as to how to demultiplex their samples based on their chosen design. Our results provide actionable insights that can assist researchers in improving cost-effectiveness and efficiency, to make the most of single-cell genomics technologies.