RNA sequencing has been widely used to determine gene expression profiles of diverse tissues, cell types, developmental stages and diseases. Most of these studies are based on population analyses using thousands of cells. Such studies, however, disguise the potentially significant biological variations among individual cells. To overcome this limitation, single-cell RNA-seq is emerging as a powerful approach to characterize gene expression heterogeneity within phenotypically identical or complex cell populations and in rare cell types.

We developed a simple and robust single-cell, low input RNA-seq workflow to generate full-length cDNA libraries from as few as 3 ng of total RNA. The workflow involves reverse transcription (RT) of RNA using NEBNext Single Cell/CTCF-baited mRNA Library Prep Kit (Catalog #E05310). This kit features the library preparation protocol, which was previously developed and validated for high-quality single-cell RNA-seq analysis. The resulting libraries can be immediately used for downstream analysis, including normalcy and reproducibility. Overall, the workflow demonstrates high levels of reproducibility and consistency when used on different cell types and samples, including single-cell datasets from mouse mammary epithelial cells differentiated through developmental stages.