Development of a novel, probe-based and instrument-free method for ultrahigh-throughput single-cell gene expression analysis, TempO-LINC

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Single cell gene expression assays are critical tools for identifying functional subtypes of cells, as well as changes within cells resulting from development, disease or treatment. However, most current methods are expensive, low in sample throughput, rely on reverse transcription and lack the sensitivity to consistently measure low expressed genes from single cells - preventing measurements of many key biomarkers and molecular pathways. Another problem with existing single-cell sequencing methods is that many of the cell-associated reads either fail to map to transcripts or map to uninformative ribosomal and mitochondrial genes. The aforementioned limitations have contributed to most single-cell sequencing studies being restricted to small numbers of samples and have prevented their wider scale adoption in biopharma, clinical, translational and applied settings.

Here we report on the development and performance of a novel combinatorial split-pool-based targeted sequencing workflow, TempO-LINC, that adds cell-identifying molecular barcodes onto high-sensitivity gene expression probes within fixed cells. All probes within the same fixed cell receive an identical barcode, enabling the reconstruction of single-cell gene expression profiles across more than 50,000 cells per run. TempO-LINC multiplexes 48, 2,000 cell samples per run, has a simple workflow that is amenable to automation and has a higher gene detection rate than existing single-cell platforms. We also will show that TempO-LINC has an observed doublet rate of less than 0.5% for 10,000 cells. Critically, TempO-LINC reduces sample sequencing costs and although we will show the assay accurately profiles the whole transcriptome (23,000 transcripts), it can also be targeted to measure only genes/pathways of interest – dramatically reducing sequencing costs yet further. All of these features position TempO-LINC as a potential disruptive technology that researchers, clinicians and drug developers can use for new large-scale applications/studies using single-cell sequencing across thousands of samples while obtaining improved data quality.