TECHNOLOGY

A drop in single-cell challenges

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Single-cell high-throughput RNA sequencing (scRNA-seq) approaches are providing unprecedented insights into the constituent cell types and intercellular heterogeneity underlying various tissues. However, one limitation is that the cost or labour intensiveness of current approaches for generating single-cell sequencing libraries using valve-based microfluidic chips or microplates has typically restricted analyses to hundreds of single cells. Two new papers report a droplet-based microfluidics approach for scRNA-seq that is readily scalable to many thousands of cells.

Klein *et al.* and Macosko *et al.* devised a microfluidics system in which fluid flows are precisely controlled to generate nanolitre aqueous reaction droplets surrounded by oil, which can then be channelled, fused and manipulated. In both approaches, single cells are individually encapsulated in droplets, together with a particle containing millions of copies of a barcoded primer (a different barcode sequence per droplet) and additional reagents. After cell lysis, the barcoded primers capture transcripts released within that droplet and are then used for reverse transcription and sequencing library preparation. High-throughput sequencing is carried out on pooled samples, and the cell-of-origin

of transcripts is inferred from the barcode sequence.

Although broadly comparable, the approaches in the two studies have some distinctions. For example, in the method of Klein *et al.*, termed inDrop (for 'indexing droplets'), particles consist of a hydrogel microsphere scaffold onto which presynthesized DNA barcode sequences are assembled, and sample pooling occurs after in-droplet reverse transcription. By contrast, in the approach of Macosko *et al.*, named Drop-seq, the particles are beads on which DNA barcodes are synthesized nucleotide-by-nucleotide, and samples are pooled before the reverse transcription step.

Both teams used mixtures of mouse and human cells to optimize conditions for achieving high-purity libraries from single cells; they identified initial cell dilutions that minimize cell doublets and RNA cross-contamination between droplets, such that ~99% of sequences associated with a given barcode were from a single species. In further technical tests, Klein *et al.* and Macosko *et al.* estimated that their methods capture 7.1% and 12.8%, respectively, of mRNAs in each cell, which is within the range of capture efficiencies of some existing scRNA-seq protocols.

For a biological application, Klein *et al.* assessed transcriptional heterogeneity in ~11,000 single

mouse embryonic stem cells (ESCs). In cultures of pluripotent ESCs and those undergoing differentiation, they identified rare subpopulations of cells and characterized their underlying gene expression profiles. Macosko *et al.* analysed ~45,000 cells from the mouse retina and found that the large number of cells allowed a refinement of known cell-type classifications and enabled the discovery of marker genes that could distinguish otherwise similar populations of retinal amacrine cells.

These droplet-based microfluidics approaches are adaptable for additional applications, such as genomic DNA sequencing, and are readily scalable to even greater cellular throughput. For example, Macosko *et al.* synthesized >16 million distinct barcode sequences, and could generate 10,000 single-cell sequencing libraries in 12 hours at ~6.5 US cents per cell, which they estimate is a >100-fold time and cost advantage over existing methods. Such savings in library preparation, in addition to ongoing reductions in the costs of sequencing, are poised to make genomic and transcriptomic analyses of many thousands of cells a routine undertaking in order to provide a comprehensive characterization of heterogeneous cell systems and tissues.

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ORIGINAL RESEARCH PAPERS

Klein, A. M. *et al.* Droplet barcoding for single-cell transcriptomics applied to embryonic stem cells. *Cell* **161**, 1187–1201 (2015) | Macosko, E. Z. *et al.* Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. *Cell* **161**, 1202–1214 (2015)