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Methods for multiplexing single-cell multi-omics

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Advances in single-cell multi-omics assays that co-detect two or more modalities enable a new frontier in resolving cellular heterogeneity and gene regulation.

The cooperative and interconnected regulation of the epigenome, transcriptome and proteome define the vastness of cell states in complex organisms[1]. A primary limitation of multi-omics assays has been cell throughput (that is, number of cells profiled), but two new technologies reported in *Nature Methods*, SUM-seq[2] and UDA-seq[3], overcome this limitation by establishing new frameworks for scalable profiling and sample multiplexing. Together, these complementary innovations offer a preview of the coming decade in single-cell genomics profiling.

Both SUM-seq[2] and UDA-seq[3] build upon a foundation of partitioning cells into oil-emulsion droplets via microfluidic technologies. In standard droplet-based assays, cell suspensions for microfluidics are highly diluted, ensuring most oil droplets contain 0 cells or 1 cell to avoid cell doublets that obscure downstream analyses[4]. However, an emerging modification for microfluidic-based profiling, termed combinatorial indexing, appends a complementary barcode to the droplet-based partitioning, scaling up cell barcoding by 1–2 orders of magnitude. Conceptually, droplet-based combinatorial indexing assays purposefully overload the microfluidic device with cells or nuclei to increase throughput and minimize the molecular biology reagents wasted in a standard reaction. An individual cell barcode is realized by combining the droplet emulsion oligonucleotide barcode with an additional source, typically a well-specific barcode. Examples of this strategy include poly(A)-targeted indexing primers with scRNA-seq[5], custom barcoded Tn5 transposase with droplet-based scATAC-seq[6] and splint oligos that extend proteo-genomic barcodes[7].

Building on these concepts of droplet-based combinatorial indexing, both SUM-seq[2] and UDA-seq[3] similarly use a two-index system for scalable barcoding (Fig. 1). While SUM-seq employs a pre-indexing strategy before the droplet partitioning, UDA-seq introduces a post-indexing step (Fig. 1). In either case, an individual cell is defined by the combination of barcodes added inside and external to the droplet partition. Irrespective of the barcoding sequence, the key assumption is that the groups of cells marked similarly in one barcoding step are randomly split in the second, resulting in distinct combinations of barcodes that discriminate nucleic acids in single cells.

In SUM-seq[2], the pre-index is introduced in two complementary steps. First, Tn5 transposase loaded with well-specific oligos generates barcoded accessible chromatin fragments (Fig. 1). Next, corresponding barcoded oligo(dT) primers are added with reverse transcriptase to allow the barcoding of mRNAs for transcriptome profiling. Following this up-front indexing for two modalities, up to 1.5 million nuclei are pooled and then overloaded onto the microfluidics chip, encapsulating multiple nuclei in one droplet. Here, a second barcode from the oligo-barcoded bead contained in the emulsion is appended to the chromatin fragment or mRNA transcript.

Critically, under the design of SUM-seq, the first barcode can be used to reidentify each cell from its corresponding sample, allowing sample multiplexing. The scale and multiplexing capabilities of SUM-seq were demonstrated by wide-ranging applications, including studying the temporal epigenetic dynamics of macrophage M1 and M2 polarization across five time points for two distinct polarization trajectories and resolving the variable regulatory landscape of cytokine-stimulated primary T cells across 36 conditions[2]. Together, these applications highlight the potential to encode dozens of perturbations that can be measured in a parsimonious assay with minimal batch effects.

As a complementary approach, UDA-seq[3] first barcodes via droplet partitioning with overloading of up to 1 million cells for the first indexing event (Fig. 1). After observing that cells and nuclei remain intact after release from droplets, the authors then aliquot the emulsion into plates for a second indexing step by well-specific PCR. Various multi-omics modalities are compatible with this approach, including accessible chromatin with mRNA transcripts as well as variable lymphocyte receptor sequences with mRNA levels. We note that a third contemporaneous method, overloading and unpacking (OAK)[8], similarly introduces a post-indexing approach, corroborating the design of this assay.

As applications of UDA-seq[3], the authors profile 35 frozen kidney samples containing tissues from healthy donors and individuals diagnosed with different metabolic or immune-mediated kidney diseases. Further, the authors delineate age-dependent diversity in TCR clonotypes by profiling multiplexed samples from 38 distinct donors. The authors recovered rare populations in both applications, highlighting the opportunities enabled by multi-omic and scalable profiling. Notably, as all samples were mixed before the initial round of barcoding in UDA-seq, the reidentification of donors required genetic demultiplexing analyses, which are feasible for distinct donors (as shown). However, this approach would not be compatible with the isogenic models analyzed by SUM-seq as the original multiplexed conditions are not encoded in the barcoding strategy.

Though standard quality control metrics (for example, unique molecular identifiers per cell) were diminished compared to those of single modality assays, both methods were shown to be technically sound with sufficient data quality to infer complex cellular relationships across heterogeneous cell models. This result holds with trends in the single-cell field, where insights have been driven by profiling exponentially more cells with less sequencing depth per cell compared to early single-cell genomics technologies. Further, both approaches were built upon commercially available reagents, library kits and

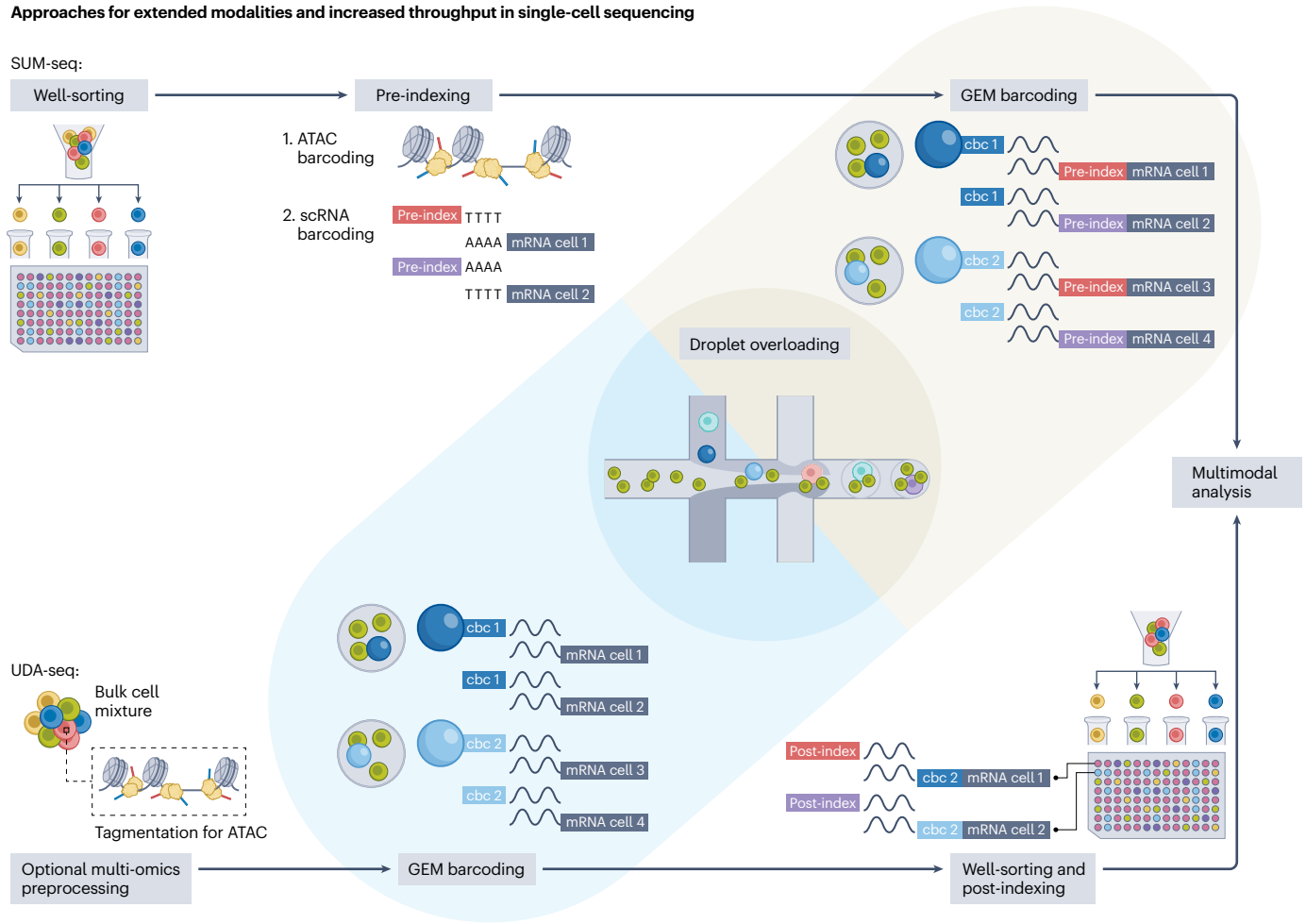

Fig. 1 | Approaches for multiplexing single-cell multi-omics. Both SUM-seq and UDA-seq combine GEM (10x Genomics) barcoding, providing a cell barcode (cbc) with an additional indexing step to achieve an individual combinatorial index for each cell, enabling droplet overloading.

protocols, permitting streamlined adoption in new laboratories. The compatibility with these commercial products is also likely to enable further methods development on top of these innovations, including layering additional modalities, including protein quantifications and somatic mutations for lineage tracing[9].

As both SUM-seq and UDA-seq are technically feasible, these multiplexed and high-content methods are likely to be widely adopted in future studies. In particular, we envision scalable single-cell screens, spanning epigenetic, genetic and chemical perturbations, being embedded in emerging multi-omic perturbation frameworks[10] and accelerated by these new methods. For example, CRISPR-based genetic and epigenetic screens directly benefit from the multiplicative gains in cell coverage unlocked by SUM-seq and UDA-seq. Indeed, perturbation screens were featured by both assays in heterogeneous settings, including differentiating human induced pluripotent stem cells profiled by SUM-seq[2] and cancer cell lines via UDA-seq[3]. As these scalable methods enable surveying a large perturbational space with multimodal readouts, causal relationships between highly correlated features that underlie cellular behavior may now be resolved via the multiplexed depth and multimodal breadth.

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References

1. Baysoy, A., Bai, Z., Satija, R. & Fan, R. *Nat. Rev. Mol. Cell Biol.* **24**, 695–713 (2023).
2. Lobato-Moreno, S. et al. *Nat Methods* https://doi.org/10.1038/s41592-025-02700-8 (2025).
3. Li, Y. et al. *Nat Methods* https://doi.org/10.1038/s41592-024-02586-y (2025).
4. Chow, A. & Lareau, C. A. *Trends Biotechnol.* **42**, 1379–1395 (2024).
5. Datlinger, P. et al. *Nat. Methods* **18**, 635–642 (2021).
6. Lareau, C. A. et al. *Nat. Biotechnol.* **37**, 916–924 (2019).
7. Hwang, B. et al. *Nat. Methods* **18**, 903–911 (2021).
8. Wu, B. et al. *Nat. Commun.* **15**, 9146 (2024).
9. Mimitou, E. P. et al. *Nat. Biotechnol.* **39**, 1246–1258 (2021).
10. Metzner, E., Southard, K. M. & Norman, T. M. *Cell Syst.* **16**, 101161 (2025).

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Competing interests

The authors declare no competing interests.