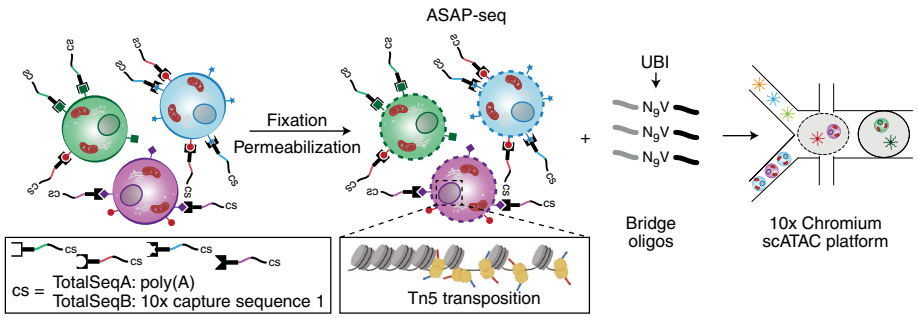
EPIGENETICS

Arsenal of single-cell multi-omics methods expanded

Chromatin accessibility, proteins levels and other features measured from the same cells.

Single-cell technologies unveil intercellular heterogeneity in various biomolecules. Moving beyond single-modal technologies, the recently burgeoning single-cell multimodal approaches achieve the feat of simultaneously measuring two or more molecular features from the same cells. In 2017, Peter Smibert, then at the New York Genome Center, and colleagues published CITE-seq, which combines single-cell RNA sequencing and multiplexed measurement of protein levels by using oligonucleotide-labeled antibodies. Not long after that, the team began thinking about applying a similar strategy to scATAC-seq (single-cell assay for transposase accessible chromatin by sequencing). "We had this tunnel vision that ATAC-seq was an assay on nuclei, so had been messing about trying to detect nuclear proteins together with ATAC," says Smibert. Despite indications that the strategy was working to some extent, the team moved on to other projects because of a dearth of antibody reagents.

This abandoned idea was not resurrected until Smibert and his colleague Eleni Mimitou listened to a talk given by Caleb Lareau, then at the Broad Institute of MIT and Harvard and Harvard Medical School. Lareau spoke about mtscATAC-seq, a method he co-developed with Leif Ludwig and other colleagues for jointly profiling single-cell mitochondrial DNA (mtDNA) mutations and chromatin accessibility. "We realized that we could essentially do mtscATAC, but by staining the cells with oligo-labeled antibodies before fixing and permeabilizing, we would be able to get surface protein information paired with chromatin accessibility." Instead of designing specific antibody–oligo reagents to be compatible with scATAC-seq, Smibert and colleagues used a 'bridge oligo' as a template to extend the antibody tag in the first amplification cycles. After annealing to the bead-derived barcoded oligo, the products undergo linear amplification in parallel with accessible chromatin fragments. Such a strategy makes it possible to use existing antibody conjugates and lowers the barrier to entry to other groups wanting to use the method, notes Smibert. "From there, we got in touch with Leif and Caleb to see if they were interested in collaborating, and together, we looped in Kelvin Chen for applying ASAP-seq to functional perturbations, and things moved very quickly."



Schematic of the cell processing steps of ASAP-seq. TotalSeq reflects products by BioLegend; Tn5 is a transposase; UBI, unique bridging identifier; N_9V, UBIs; colored asterisks, barcoded beads. Credit: Reprinted with permission from E. P. Mimitou et al. *Nat. Biotechnol.* https://doi.org/10.1038/s41587-021-00927-2, Springer Nature

The method the team thus developed, ASAP-seq (ATAC with Select Antigen Profiling by Sequencing) can simultaneously collect information about chromatin accessibility, abundance of hundreds of cell surface and intracellular proteins, and mtDNA variants for thousands of single cells. To incorporate even more features, the team further developed DOGMA-seq, which, in addition to the above three data types, also quantifies RNA levels. Although DOGMA-seq was motivated by combining CITE-seq and 10x Genomics' recent Multiome product, "the cell fixation needed to input whole cells instead of nuclei into the scATAC and Multiome products involves crosslinking macromolecules and can have a negative impact on RNA quality," notes Smibert, saying, "Optimizing the fixation conditions to tune cell surface marker and mtDNA detention while maintaining high quality of RNA capture was a major challenge while developing DOGMA-seq."

The team applied ASAP-seq, DOGMA-seq and other high-throughput approaches to investigate human hematopoiesis, immune cell stimulation, and effects of multiplexed CRISPR perturbations in T cells. The rich data reveal complex interrelationships between different layers and refine identification of cell types and states.

The first versions of these methods may benefit from improvements to increase data quality, as well as simplify their usage, notes Smibert. Other directions for future development include increasing scale while reducing costs per cell and combining these approaches with perturbation approaches. "In terms of applications, these tools are really universally applicable, and we foresee them to be widely used across the different fields of biomedical research and in the future possibly also routine clinical application for diagnostic purposes."

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