> “combining single-cell RNA sequencing (scRNA-seq) with CRISPR-based lineage tracing”

Key aspects of developmental biology include the diversity of cell types that make up an organ or organism and the developmental lineage history of those cells. These aspects have typically been studied separately, but three new papers report methods for dissecting transcriptomic cell phenotypes and lineage history simultaneously by combining single-cell RNA sequencing (scRNA-seq) with CRISPR-based lineage tracing.

The CRISPR–Cas9 genome-editing system has recently been adapted for cell lineage tracing. In a typical implementation, a barcode array is engineered into the genome of an organism; then, at an early developmental stage, Cas9 is co-injected with guide RNAs (gRNAs) to direct its nuclease activity to the barcode array. During the transient period of Cas9 activity, the barcodes are cumulatively cut and repaired in variable ways in different cells. When the barcode sequences are later analysed at a chosen developmental time point, lineage relationships among cells can be inferred from patterns of shared Cas9-induced mutations.

Three recent studies extend this approach by leveraging the cell-type information provided by scRNA-seq to examine zebrafish development. Alemany et al. developed the method ScarTrace, in which transgenic genomic integration of eight tandem copies of a *GFP* gene is targeted following embryonic injection of either Cas9 protein or Cas9 RNA with a *GFP*-targeted gRNA.

For scRNA-seq analysis of the resultant tissues, the team used a sorting and robot-assisted transcriptome sequencing (SORT-seq) protocol. Although the Cas9-edited *GFP* sequences (‘scars’) are typically expressed in cells and hence can be read out from the transcriptome, SORT-seq incorporates a nested PCR step to read out the scar sequences from the remaining genomic DNA in the single cells, thus minimizing the loss of lineage information for cells in which *GFP* expression is absent.

The investigators analysed the clonal histories of various tissues — including the haematopoietic system, adult brains and eyes, and regenerating fins — to provide various developmental insights, such as the numbers and types of progenitor cells that are precursors of specific subsets of differentiated cells.

Spanjaard et al. report a conceptually similar approach termed lineage tracing by nuclease-activated editing of ubiquitous sequences (LINNAEUS). The method involves embryonic injection of Cas9 protein and a gRNA that targets 16–32 integrations of a red fluorescent protein (*RFP*) gene spread throughout the genome. The rationale for using genomically dispersed target sequences is to avoid deletions between nearby arrayed copies, which could potentially remove informative copies between them.

LINNAEUS was applied to zebrafish larvae, plus adult heart, liver, pancreas and telencephalon. The team performed scRNA-seq using droplet-based microfluidics, and the mutated *RFP* sequences were read out from the transcriptome, which was split into a library for transcriptome-wide analysis and one for targeted analysis of *RFP* sequences.

Beyond the experimental progress, Spanjaard et al. highlight the bioinformatics challenges and the need for a custom analysis pipeline, as standard phylogenetic algorithms derived from species evolution studies are suboptimal for cell lineage reconstruction, owing to the large numbers (tens of thousands) of single cells being compared and incomplete detection of lineage barcodes.

The most notable distinction of the third study is that Raj et al. specifically sought to overcome the limitation that embryonically injected Cas9 is only active for the first few hours of development. Therefore, in addition to embryonic injection of Cas9 protein and four gRNAs targeting sites 1–4 of an *RFP* barcode, the fish also transgenically encode Cas9 and five additional gRNAs targeting sites 5–9 of the *RFP* barcode, which can all be induced through heat shock at a chosen time to monitor later developmental stages.

In their method, termed scGESTALT (a single-cell derivative of the previously reported GESTALT tracing method), scRNA-seq was carried out similarly to Spanjaard et al., using droplet-based microfluidics and targeted transcriptome enrichment for barcode sequencing. Raj et al. focused their analysis on the brain and identified numerous lineage relationships among neural cell types and the likely gene expression cascades during developmental transitions.

These powerful methods can be applied to study a wide range of normal and pathological developmental phenomena, potentially also in other model organisms.

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