DEVELOPMENT

New dual-channel system records lineage in high definition

Introducing iTracer, a system for long-term measurement of lineage dynamics at high resolution.

Kunal Jindal, Sadie VanHorn and Samantha A. Morris

Lineage tracing, the process of identifying and tracking the progeny of cells, is a powerful approach for understanding complex biological processes. In developmental biology, it aids scientists in mapping the origins of different lineages within the embryo, revealing molecular factors responsible for cell fate specification. Writing in this issue of *Nature Methods*, He and Maynard et al. describe iTracer, a method for reconstructing lineage relationships from single-cell data[1].

Lineage tracing originates in the 1890s when vital dyes were used to label cells and their progeny, allowing visual tracking of cell relationships. This approach enabled the construction of fate maps, which depict the tissue types that arise from specific areas of the embryo. These original lineage tracing methods were limited to embryos that could develop in the dish under microscopic observation, precluding their use in complex, inaccessible organisms[2,3].

Recently, single-cell lineage tracing (scLT) methods have emerged that combine lineage tracing with single-cell RNA sequencing (scRNA-seq), a high-throughput assay that measures gene expression in many individual cells[4,5]. Foundationally, scLT works by uniquely labeling cells; but rather than using vital dyes, one major class of experimental methods uses heritable DNA barcodes expressed and captured as RNA transcripts to mark cells indelibly. This approach enables the parallel measurement of lineage relationships and detailed cellular characteristics in an ever-increasing number of cells.

For successful lineage tracing at sufficient resolution, unrelated cells must be labeled with unique barcodes. scLT methods either achieve the necessary barcode diversity by using a large library of static, random sequences or generate diversity via CRISPR-mediated mutation of a common sequence. Static barcoding is relatively straightforward and does not require the introduction of CRISPR editing components into the cell. However, static barcoding is typically limited to labeling cells at one or a few time points, limiting the complexity of lineage trees constructed from the data[4]. On the other hand, barcodes that can be repeatedly edited to mutate over time enable higher-resolution mapping of multi-step biological processes such as development. However, such mutable approaches suffer from a higher rate of barcode collision[6], a phenomenon where the same edits occur in two unrelated cells due to inherent bias in CRISPR-based DNA editing, a problem that increases with every round of mutation.

With iTracer, He and Maynard et al. overcome these current challenges in scLT by combining the above static and CRISPR-mutable barcoding strategies. Each iTracer barcode consists of both static, random and mutable portions. Their experimental design labels a starting population of cells with iTracer barcodes, with the static portion uniquely demarcating each labeled cell. Following cell expansion, sibling cells that are derived from a single cell share identical static barcodes. These groups of clonally related cells are referred to as a 'barcode family'. CRISPR editing of the mutable portion is then induced at a later time point to demarcate subclones within each clone (barcode family), termed 'scar families'. Finally, cells are profiled with scRNA-seq at the end of the experiment to measure single-cell gene expression and capture lineage information (Fig. 1). As the lineage identity of each cell is defined by a combination of its static and mutable barcodes, the probability of barcode collision is significantly reduced.

The iTracer team used this versatile design to study the lineage dynamics of cerebral organoid development from induced pluripotent stem cells (iPSCs). Measuring the spatial distribution of clonally related cells can elucidate developmentally relevant cell migration and tissue patterning. By clustering cells into different neuronal cell types based on their gene expression profiles and overlaying lineage information, they observed the accumulation of clonally related cells into distinct clusters resembling different brain regions. These results were also directly validated with four-dimensional live microscopy and spatial transcriptomics.

Another powerful application of iTracer is in determining the timing of fate restriction. During development, stem cells give rise to a variety of differentiated cell types. Their decision to commit to a particular cell fate is defined by a combination of cell-intrinsic factors and extra-cellular signals. Studying this process of fate restriction can reveal key genes and molecules driving commitment to distinct lineages. By varying the timepoint for CRISPR editing of the mutable iTracer barcodes during organoid formation and monitoring the change in composition of scar families, the iTracer team defined a coarse window for fate restriction. If scarring is performed before fate restriction, each scar family will consist of a highly diverse population as cells have not yet committed to a discrete fate outcome. Alternatively, if scarring is performed after fate restriction, most scar families will consist of one or a few cell types. Paired with detailed single-cell gene expression profiling, the spatial and temporal aspects of iTracer qualify it as an important tool for creating high-resolution multidimensional fate maps for developing embryos of complex animals.

Despite its versatility, there are some technical limitations to iTracer. While barcodes are detected in most starting iPSCs, less than 50% of the cells retain detectable barcodes after organoid formation. The authors attribute this to gene silencing, wherein the barcodes are sequestered within parts of the genome that prevent their detection, and dropout, which refers to the limited ability of scRNA-seq to measure expressed transcripts. Another recent publication[7] offers a lineage-tracing method that counters gene silencing by inserting a DNA barcode, termed 'CHYRON', into a genomic safe-harbor locus and flanking it with insulator elements that impede molecular silencing of genes. However, CHYRON is not compatible with single-cell sequencing. In addition to development, scLT has the potential to support novel discoveries in other fields of biology. For example, a recent paper[8] applied scLT to a

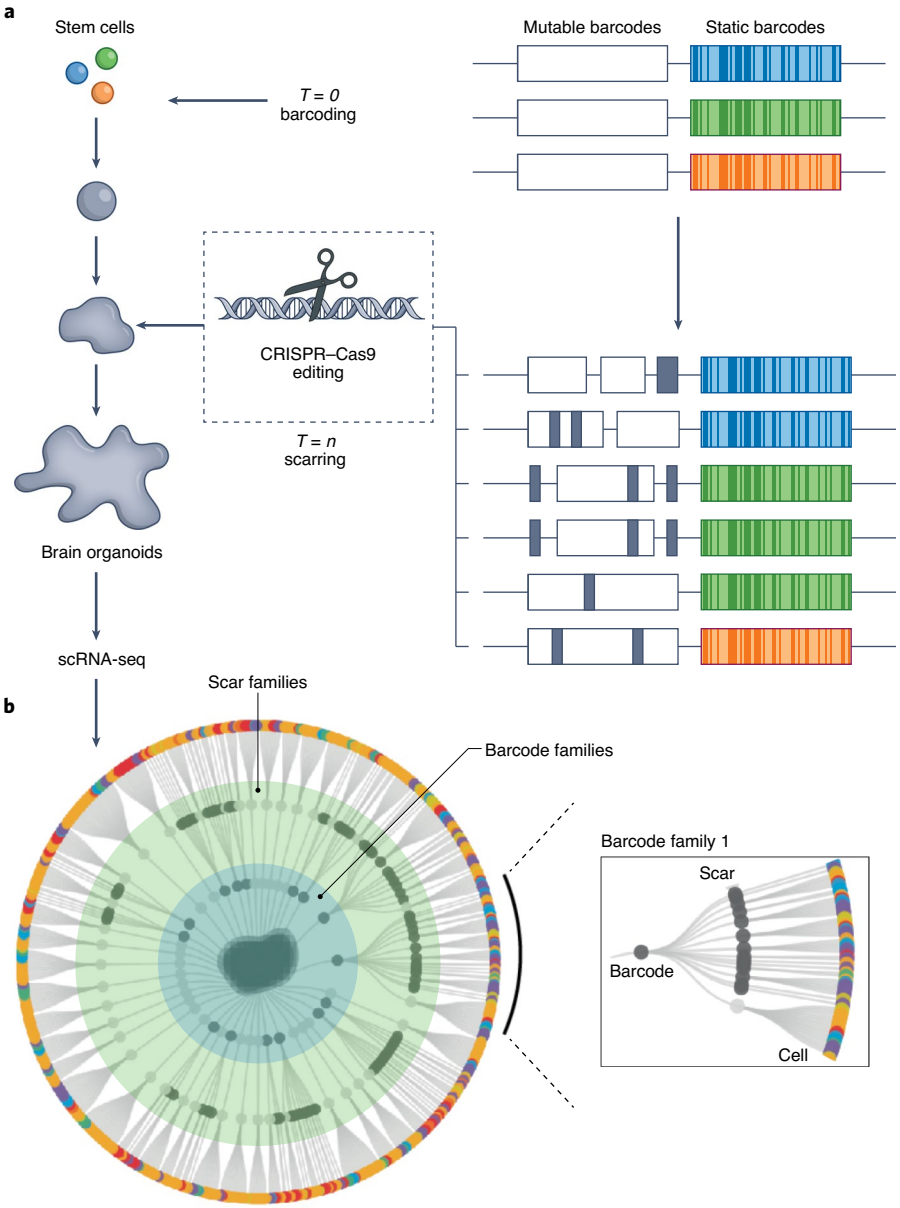

Fig. 1 | High-resolution recording of single-cell lineages. He and Maynard et al.[1] report the development of iTracer, a lineage-tracing system that uses a combination of static and mutable barcodes to track single-cell lineages and use it to study cerebral organoid development. Each iTracer barcode consists of a unique static portion and a constant mutable portion. **a**, As a first step, stem cells are labeled with the iTracer library such that each cell acquires a unique static barcode signature, allowing tracking of starting clones. Following this, cells are induced to form cerebral organoids. During this process, CRISPR–Cas9 editing of the mutable barcode is induced at a time point of interest to achieve an additional layer of lineage tracing within each clone. **b**, At the end of the experiment, cells are profiled using scRNA-Seq and iTracer barcode sequences are used to construct lineage trees. Cells that share the same static barcode signature and grouped into barcode families. Within each barcode family, cells that share the same mutable barcode edits are grouped into scar families. Adapted from ref. [1], Springer Nature Ltd.

mouse model of pancreatic cancer. Lineage analysis allowed the authors to identify transcriptional states associated with cancer metastasis and compare expansion between metastatic and non-metastatic clones. They used an inducible scLT system consisting of both static and mutable components, similar to iTracer.

iTracer and other scLT systems mark an exciting new chapter in the field of lineage tracing. Advances in single-cell techniques and DNA-editing tools are driving progress in the field at a rapid pace. Recently, new methods of DNA editing such as base editing and prime editing have emerged. Due to their alternative mechanisms of action, these methods are not susceptible to large deletions during editing that lead to loss of lineage information, as occurs with current CRISPR-based approaches. These emerging editing approaches could enable new mutable scLT systems that continuously accrue edits over several cell divisions, allowing for higher-resolution lineage-tracing experiments. Furthermore, the development of new single-cell modalities is helping scientists measure cellular characteristics beyond gene expression, such as chromatin accessibility and surface proteins. Extension of scLT to these new single-cell methods could afford a truly multidimensional view into how cell identity is established and maintained. Finally, efforts to simplify data analysis and streamline data sharing have taken center stage amongst the global single-cell community. Extension of these ideas to scLT could help build end-to-end lineage analysis pipelines and unified data formats that can accommodate the diverse scLT methods that currently exist, rendering them more accessible to a broader scientific community. ❐

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Published online: 30 December 2021
https://doi.org/10.1038/s41592-021-01340-y

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Acknowledgements

S.A.M. is supported by an Allen Distinguished Investigator Award (through the Paul G. Allen Frontiers Group), a Vallee Scholar Award, a Sloan Research Fellowship and a New York Stem Cell Foundation Robertson Investigator Award. S.V. is supported by National Institutes of Health grant 5T32GM007067-46.

Competing interests

The authors declare no competing interests.