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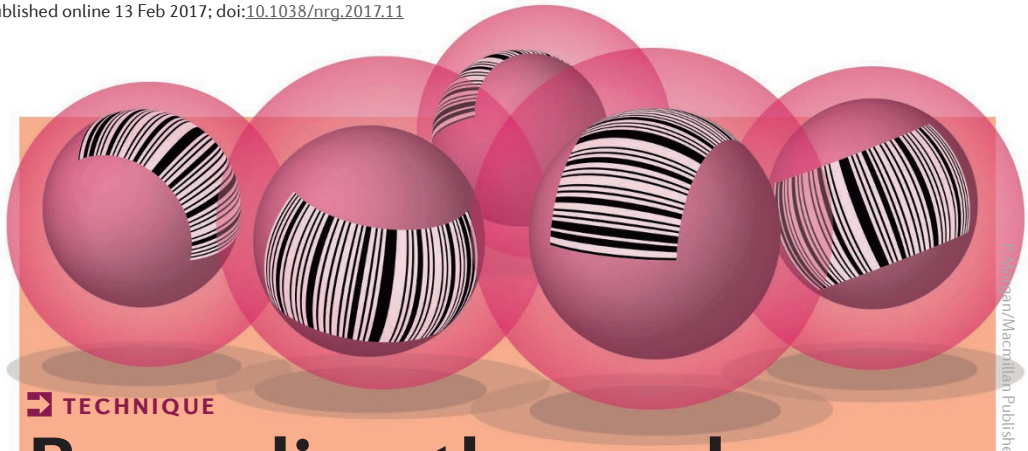

TECHNIQUE

Barcoding the nucleus

> higher throughput should encourage replication of experiments

Single-cell sequencing technologies have had a major impact on our understanding of cell heterogeneity in a range of biological contexts. However, the requirement for physically isolated cells limits the number of cells that can be analysed and the types of studies that can be done. Now, two new reports in *Nature Methods* tackle this problem by applying single-cell combinatorial indexing (SCI) to whole-genome sequencing (SCI-seq) and to Hi-C (sciHi-C).

SCI involves sequential labelling of nucleic acids in intact nuclei, enabling individual nuclei to be discriminated by a unique two-index identifier. Thousands of nuclei can be processed in a single workflow, obviating the need for disaggregation and isolation of single cells. The potential of SCI to improve single-cell analysis has been demonstrated previously for sciATAC-seq, a chromatin accessibility assay.

Now, Vitak *et al.* have developed SCI-seq, which incorporates SCI into a modified single-cell genome sequencing workflow. Crucially, SCI-seq includes a nucleosome depletion step, which is essential to minimize potential bias, before using Tn5 transposase to randomly barcode intact nuclei with one of 96 indexed sequencing adaptors. Differentially barcoded nuclei are then mixed and sorted into size-restricted groups of up to 25 nuclei to increase the likelihood that each nucleus has a different first-round index. Finally, each group is differentially barcoded by indexed PCR, generating a unique two-index combination for each nucleus. While 'collisions' (more than one nucleus with the same combinatorial index) do occur, the collision rate can be controlled by regulating the number of nuclei pooled for second-round indexing. Current coverage and uniformity are sufficient for studying copy number variation (CNV) and aneuploidy, but not single-nucleotide variation (SNV). The authors used SCI-seq to generate 16,698 single-cell libraries from diverse starting samples including cell lines, archived tissues and fresh and frozen primary tumour samples; 5,395 of these were sequenced to a depth sufficient for CNV calling at a resolution of 250 kb.

In a separate study, Ramani *et al.* applied combinatorial labelling to the study of chromosome conformation using sciHi-C. In sciHi-C, intact nuclei are restriction digested and then sorted into 96-well plates for barcoding with indexed double-stranded bridge adaptors. Nuclei are then pooled, proximity ligated, and up to 25 nuclei sorted into each well of a second 96-well plate; if appropriate, fewer cells can be pooled to decrease the collision rate. At this point, the nuclei are lysed and barcoded with an indexed Y-adaptor. After sequencing the junctions, single-cell contact maps can be constructed for each nucleus based on its unique two-index identifier. The authors used sciHi-C to generate 10,696 single-cell contact maps with at least 1,000 unique contacts. Of these, 3,515 maps had more than 10,000 unique contacts. For comparison, in a previously published single-cell Hi-C study only ten cells reached a similar coverage cut-off threshold.

SCI-seq and sciHi-C, together with sciATAC-seq, provide an impressive suite of accessible high-throughput single-cell methods for probing the genome. By removing the requirement for expensive microfluidic equipment, these SCI-based methods have the potential to democratize the single-cell field, opening up the technology to a wider range of laboratories. But perhaps most importantly for the field, the lower cost and higher throughput should encourage replication of experiments, ultimately leading to more consistent data and more robust conclusions.

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ORIGINAL ARTICLES Vitak, S. A. *et al.* Sequencing thousands of single-cell genomes with combinatorial indexing. *Nat. Methods* http://dx.doi.org/10.1038/nmeth.4154 (2017) | Ramani, V. *et al.* Massively multiplex single-cell Hi-C. *Nat. Methods* http://dx.doi.org/10.1038/nmeth.4155 (2017)

FURTHER READING Gawad, C., Koh, W. & Quake, S. R. Single-cell genome sequencing: current state of the science. *Nat. Rev. Genet.* **17**, 175–188 (2016) | Schwartzman, O. & Tanay, A. Single-cell epigenomics: techniques and emerging applications. *Nat. Rev. Genet.* **16**, 716–726 (2015)