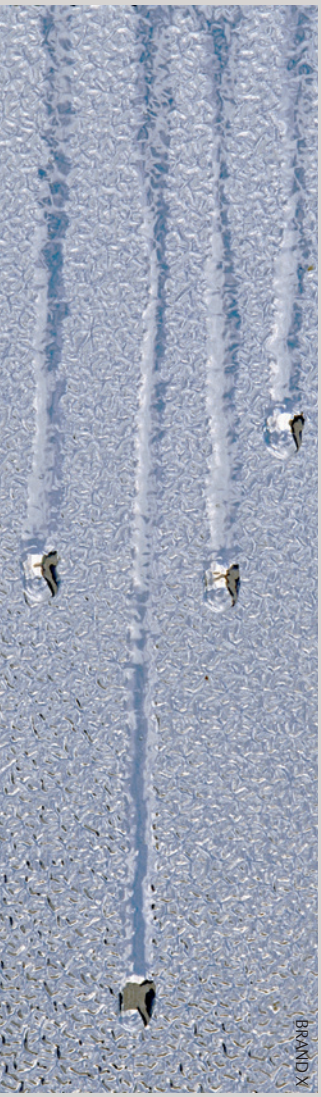
Nature Reviews Genetics | AOP, published online 27 October 2015; doi:10.1038/nrg4036



TECHNOLOGY

Dropping in on single-cell epigenetic profiles

In recent years, there has been a growing appreciation for cellular heterogeneity and the development of methods to study this phenomenon. However, the mapping of chromatin profiles in single cells has posed a substantial technical challenge. A new method harnesses a number of cutting-edge technologies — microfluidics, DNA barcoding and next-generation sequencing — to allow single-cell analysis of chromatin states.

Chromatin immunoprecipitation followed by sequencing (ChIP–seq) is commonly used to analyse chromatin states. However, applying ChIP–seq to single cells has been limited by nonspecific antibody binding during the immunoprecipitation step, which leads to experimental noise and confounds data interpretation when the amount of starting sample material is low. Rotem, Ram and Shoresh *et al.* now describe an adaptation of ChIP–seq, called Drop-ChIP, and apply it to acquire information on histone states in single cells. The researchers developed a drop-based microfluidics device, in which 50 μM-sized aqueous drops restrain single cells and act as microreactors that can be manipulated in the device. The droplets contained micrococcal nuclease to digest chromatin, making nucleosomes accessible. The investigators also created a library of unique oligonucleotide 'barcodes' enclosed in separate droplets, then combined each nucleosome drop, a barcode drop and DNA ligase, thus labelling the chromatin from each individual cell with an identifier barcode. The tagged chromatin from 100 cells was combined into pools and then subjected to ChIP, followed by next-generation sequencing. This method enables individual cells to be subsequently tracked from a combined pool and helps to limit the confounding noise associated with small numbers of input cells.

To test their approach, the researchers analysed three populations of mouse cells — embryonic stem cells (ESCs), fibroblasts and haematopoietic progenitor cells — for two types of histone mark. The team successfully separated each population of cells on the basis of their histone states, and found that the single-cell profiles correlated with known bulk chromatin profiles for each cell type. Importantly, a single-cell chromatin profile from a cell of one type could be accurately used to distinguish it from another cell type, highlighting the sensitivity of the method. However, there was a pay-off for this high sensitivity with per-cell sequencing coverage; only ~1,000 unique reads were detected per cell, corresponding to a sensitivity for peak detection of ~5%. The authors also showed that their method could identify distinct epigenetic states within a single cell population; for example, they identified three subpopulations of ESCs, one with epigenetic states corresponding to pluripotency, one with epigenetic states consistent with priming for differentiation and a third type that fell in the middle of these two states.

Although future optimization will be required to improve sequencing coverage, this method represents an important advance. Application of Drop-ChIP is expected to enable a more comprehensive exploration of cellular epigenetic heterogeneity, which will provide new insights into its role in both basic biology and disease states such as cancer.

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The author declares no competing interests.

ORIGINAL RESEARCH PAPER Rotem, A., Ram, O. & Shoresh, N. *et al.* Single-cell ChIP-seq reveals cell subpopulations defined by chromatin state. *Nat. Biotechnol.* http://dx.doi.org/10.1038/nbt.3383 (2015)

FURTHER READING Schwartzman, O. & Tanay, A. Single-cell epigenomics: techniques and emerging applications. *Nat. Rev. Genet.* http://dx.doi.org/10.1038/nrg3980 (2015)