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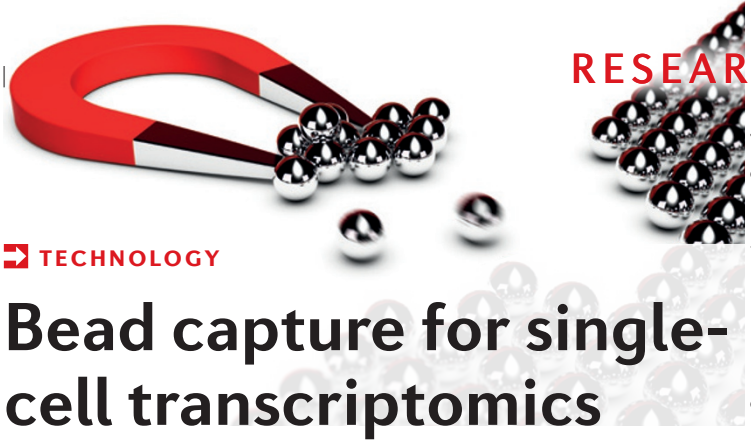

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TECHNOLOGY

Bead capture for single-cell transcriptomics

Single-cell approaches are required to gain deep biological insights into heterogeneous cell samples. Two common strategies for single-cell gene expression profiling are flow cytometry (in which a small number of cell surface marker proteins are characterized in millions of cells) and transcriptome-wide RNA sequencing (RNA-seq) on isolated cells (where the costs of instrumentation, reagents and sequencing typically limit the analyses to a few hundred cells). A new report describes a bead-based approach to characterize a custom subset of the transcriptome across thousands of cells, offering a complementary method that is scalable to a large number of samples.

For single-cell transcriptomic analyses, Fan *et al.* devised the CytoSeq method using a microfabricated surface containing up to 100,000 microwells. A single-cell suspension and a set of DNA-labelled magnetic beads are deposited at a density that facilitates a single cell and a single bead to co-occupy the microwells. Following cell lysis, the mRNAs from each cell hybridize to the poly(T) portions of the DNA oligonucleotides on the proximal bead. Other sequences within each bead oligonucleotide are: a universal sequence for PCR-based amplification; a sequence that identifies each bead (and hence also the cell of origin of the bound mRNAs); and a unique molecular identifier (UMI) that is variable among the thousands of oligonucleotides on each bead and that serves as a means for controlling subsequent PCR bias and for absolute transcript quantification. Following hybridization, beads and their bound transcripts are magnetically extracted from the surface, pooled, and the bulk sample is processed for cDNA synthesis, PCR-based amplification of a custom cDNA set of interest and DNA sequencing. The sequencing reads thus contain information on absolute mRNA molecule counts assigned to each gene and cell under study.

The investigators applied CytoSeq to a range of proof-of-principle applications on human haematopoietic cell samples. Characteristic expression levels of 12–111 marker transcripts were used to distinguish cell types in heterogeneous cell samples, such as cell lines mixed at defined ratios, heterogeneous normal peripheral blood mononuclear cells, and T cells at different states of activation. Notably, from 2,855 T cells, they identified 7 cells that were probably antigen specific based on the expression of interferon gamma (*IFNG*). The ability to analyse thousands of cells to identify such rare cell populations is likely to be a key strength of CytoSeq. These large cell numbers are achievable because, as the authors estimate, the cost of consumables is 2–3 orders of magnitude lower than for current commercial microfluidics-based single-cell RNA-seq approaches.

One potential limitation of CytoSeq is that analysed transcripts must be chosen in advance, thus limiting the ability to discover roles for genes that are not known to be involved in the process under study. However, cDNA-bound bulk bead samples serve as an archive of each experiment that can be re-interrogated for different gene sets, or even for whole-transcriptome amplification and analysis. Currently, the sequencing costs for whole-transcriptome characterization of thousands of single cells are still largely prohibitive, although the continually decreasing costs might make whole-transcriptome CytoSeq a highly attractive approach in the future.

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ORIGINAL RESEARCH PAPER Fan, H. C., Fu, G. K. & Fodor, S. P. Combinatorial labeling of single cells for gene expression cytometry. *Science* **347**, 1258367 (2015)
FURTHER READING Shapiro, E., Biezuner, T. & Linnarsson, S. Single-cell sequencing-based technologies will revolutionize whole-organism science. *Nature Rev. Genet.* **14**, 618–630 (2013)