A novel method to characterize genes with high-precision in single cells

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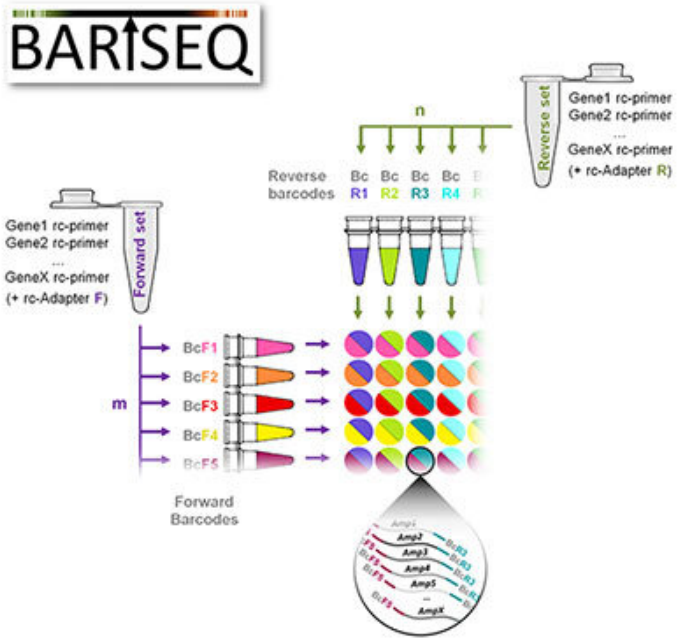

Credit: Helmholtz Zentrum München

The analysis of gene products in cells is an important tool for diagnosing disease and the design of new active substances in biological and medical research. At Helmholtz Zentrum München, a method of targeted RNA

sequencing (transcriptome analysis) has now been developed, which precisely detects the smallest amounts of gene transcripts in single cells. The method enables the identification and enrichment of individual selected molecules in a sample in order to investigate their cellular function. This makes it possible to selectively characterize genes in each cell with high precision. Their work has been published in *Genome Biology*.

Single cell RNA sequencing is based on the investigation of the molecular transcripts generated by active regions of the genome in individual cells. Depending on type and stage of development, cells activate different gene sets that are read from RNA molecules and translated into proteins. The number of mRNA molecules—also called transcripts—per gene in a given cell can inform us about their identity and their physiological response to internal or external signals. These can be diseases, aging process, environmental influences or reactions to pharmacological agents. However, the detection of genes that are only expressed in moderate to low concentrations poses a major challenge for current single cell RNA sequencing techniques. They mostly detect so-called housekeeping genes, which, unlike regulated genes, are constantly expressed.

The BART-Seq method, developed by a team around Dr. Micha Drukker, Institute of Stem Cell Research, and Ph.D. student Fatma Uzbas, addresses this problem by enriching selected transcripts for sequencing. BART-Seq stands for "Barcode Assembly foR Targeted Sequencing." Primer sets and DNA barcodes are combined, so that they can simultaneously amplify the transcripts of the genes of interest. "We have developed a novel way to index primers with DNA barcodes by a simple synthesis reaction," explains Micha Drukker. Sequenced transcripts can thus be traced back to the individual cells from which they originated. Since the analysis focuses on the selected genes, it is possible to obtain high-resolution information about these genes and thus

characterize each cell individually.

The method is inexpensive and does not require specialized and expensive instrumentation. Any research group with access to a Next-Generation Sequencing device can use BART-Seq both for single cells and for the analysis of RNA or genomic DNA bulk specimens from thousands of samples.

Together with Philipp Angerer, Nikola Müller and Fabian Theis from the Institute of Computational Biology at Helmholtz Zentrum München, Drukker's team has developed software for the design of primers and barcodes as well as for the analysis of sequencing data. In order to make the method accessible to all research groups, the software is freely available on the Internet.

Micha Drukker and his team colleagues hope that their method will become an integral part of the toolkit for basic and applied research. Projects on drug screening, such as the measurement of the reaction of cultured β cells to drugs, or precision gene-editing technology using CRISPR-Cas9 could benefit greatly from BART-Seq.

More information: Fatma Uzbas et al. BART-Seq: cost-effective massively parallelized targeted sequencing for genomics, transcriptomics, and single-cell analysis, *Genome Biology* (2019). DOI: 10.1186/s13059-019-1748-6

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