Enabling functional genomics studies in individual cells

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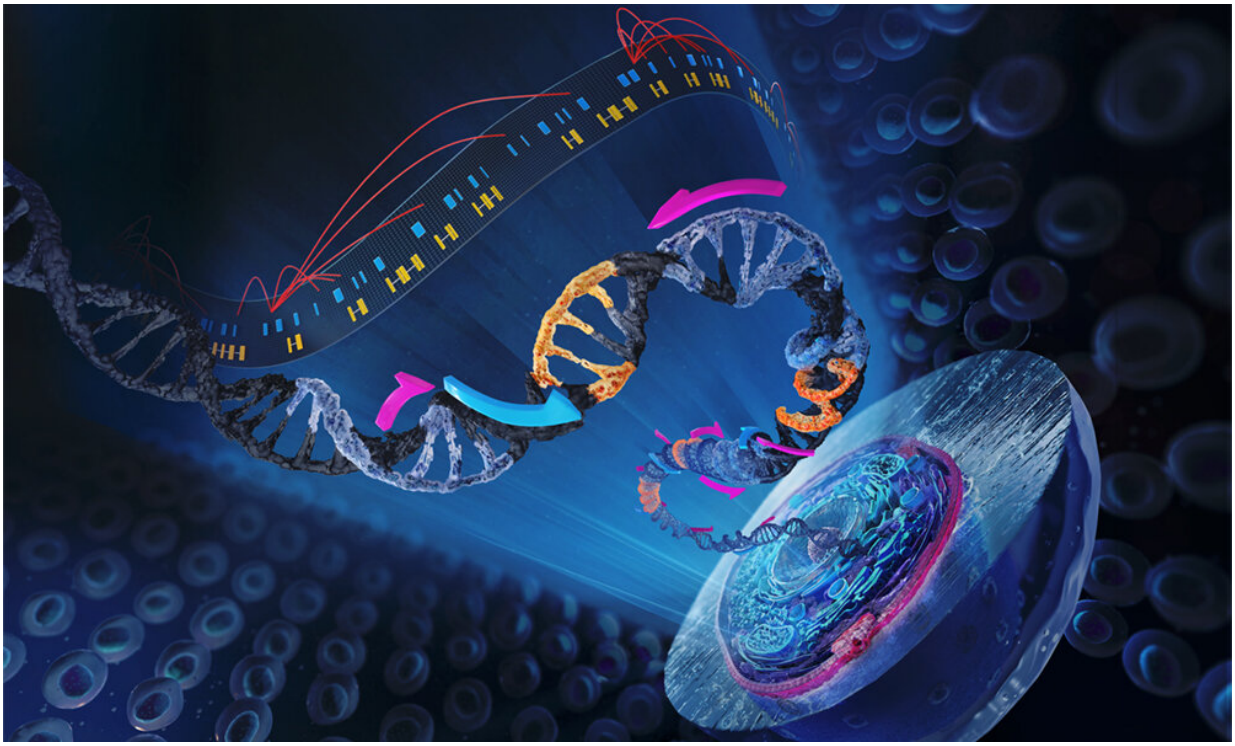

An illustration of single-cell RNA sequencing (scRNA-seq), a powerful analysis method that gives researchers detailed insights into levels of gene expression in individual cells. Credit: Tobias Wüstefeld/BlueClay Studios

Although the first complete sequence of the human genome was reported more than 15 years ago, the biological functions of large parts of the genome are not yet known. Scientists working in the field of

functional genomics try to understand these functions, and how our genome determines our individual traits and the course of diseases. High-throughput methods, such as CRISPR–Cas9 genome editing, allow scientists to study the functions of genomic elements in a systematic way. They use CRISPR–Cas9 to modify thousands of genes in a single experiment, but analyzing the biological effects of these modifications is challenging.

Single-cell RNA sequencing (scRNA-seq) is a powerful analysis method that gives researchers detailed insights into levels of gene expression in individual cells, and has been used to analyze CRISPR–Cas9 functional genomics screens. However, scRNA-seq has previously been limited in its ability to detect low levels and subtle changes of gene expression. The high cost of the method has further limited the scale of applications in functional genomics and the amount of information that could be obtained from them. To overcome these restrictions, EMBL scientists have designed a new approach for analyzing the outcome of genome-wide CRISPR–Cas9 screens, called Targeted Perturb-seq (TAP-seq). TAP-seq was developed by postdocs Daniel Schraivogel and Andreas Gschwind in Lars Steinmetz's groups at EMBL and Stanford University, respectively; by Lars Velten, now at the Centre for Genomic Regulation (CRG) in Barcelona; and by Lars Steinmetz, a group leader at EMBL and Stanford. Their paper is published in *Nature Methods*.

Overcoming limitations

Expression of a gene occurs when its DNA sequence is used as a template to create a molecule known as messenger RNA (mRNA), in a process known as transcription. The mRNA transcript can then carry out other functions, such as instructing the cell to make a protein. The number of mRNA transcripts of a particular gene is therefore a measure of that gene's activity. Instead of analyzing the expression levels of all genes across the whole genome, TAP-seq focuses on preselected sets of

genes that are relevant to the biological question being investigated. By picking out and amplifying transcripts of only a subset of genes, the method becomes much more sensitive. With TAP-seq, it's possible to analyze genes that are only transcribed into a very small number of mRNA molecules, and to detect subtle changes amounting to an increase or decrease of only one mRNA molecule per cell.

TAP-seq allows scientists to analyze up to 1,000 genes in a single experiment and is up to 50 times less expensive than previous protocols. "By providing all tools for researchers to design their own TAP-seq experiments and analyze the generated data, we want to make our method easily accessible to the wider scientific community," says Andreas Gschwind, one of the two lead authors of the study. The authors believe that TAP-seq will make scRNA-seq much more affordable and will enable new research projects in biomolecular and biomedical research.

Understanding gene regulation

For their first application of TAP-seq, the EMBL scientists used CRISPR–Cas9 to inactivate nearly 1,800 regulatory stretches of DNA, one at a time in individual cells. These DNA regions are known as enhancers, and are specialized regions in the genome that determine how strongly neighboring genes are transcribed. The scientists afterwards used TAP-seq to analyze how the perturbation of an enhancer in a cell affected gene expression. "Previously, the relationships between enhancers and genes have been analyzed mainly by looking at how chromosomes fold inside a cell's nucleus. But this doesn't tell you whether enhancers indeed affect gene activity," says Daniel Schraivogel, who shares lead authorship of the study. "With TAP-seq, we can directly measure which genes change their activity. This allows us to discover rules that describe gene regulation by enhancers more accurately than ever before."

Importantly, TAP-seq allows researchers to perform CRISPR–Cas9 screens with much higher sensitivity and on a larger scale than before. The new method increases the knowledge gained from a functional genomics screen, while also reducing the cost of the analysis. "This way, we can understand the complicated grammar of the human genome. We can test thousands of genes in an affordable two-day experiment," says Lars Steinmetz, senior author of the study. There are many opportunities to apply TAP-seq in biological research. In an upcoming project, members of the Steinmetz group are planning to use TAP-seq to analyze how gene variants affect the development of immune diseases.

More information: Daniel Schraivogel et al. Targeted Perturb-seq enables genome-scale genetic screens in single cells, *Nature Methods* (2020). DOI: 10.1038/s41592-020-0837-5

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