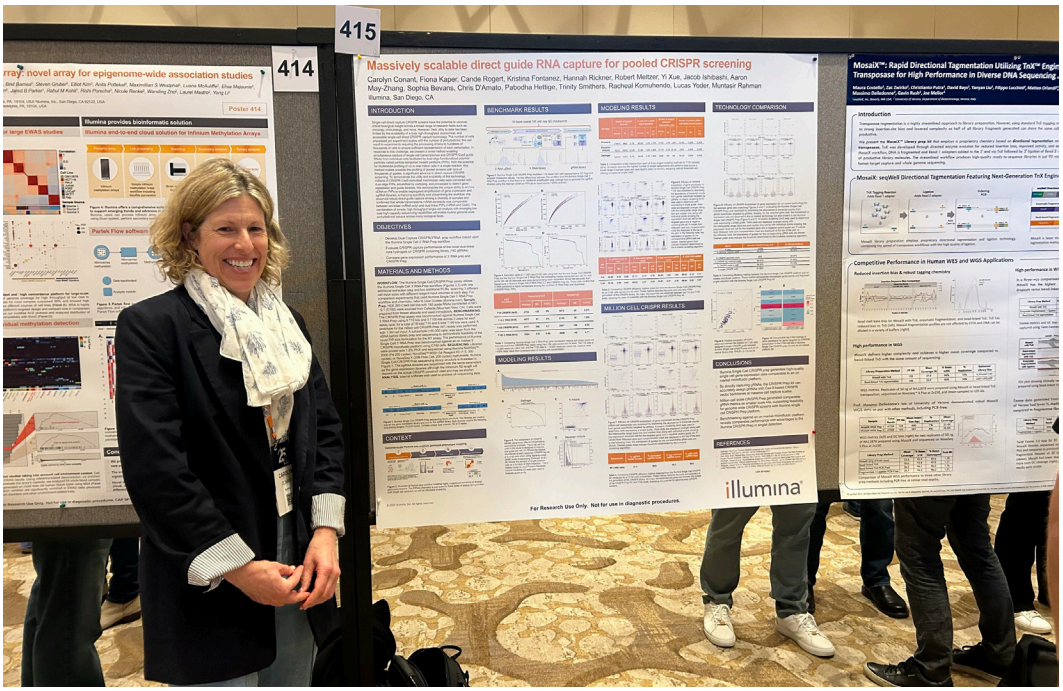

Illumina Director of Systems Integration Carolyn Conant presents the poster at AGBT 2025.

Illumina's high-throughput single-cell CRISPR prep makes gene editing a reality

At AGBT 2025, Illumina scientists present a breakthrough method for interrogating the gene expression of individual cells

EVER SINCE THE GENE EDITING technique CRISPR-Cas9 was discovered in 2012,[1] researchers have been enthusiastic about its potential to treat human diseases. For the first time, CRISPR-Cas9 offered a precise, targeted way to modify genes: Researchers design specific segments of RNA in the CRISPR portion of the CRISPR-Cas9 system that targets a gene of interest through sequence complementarity. That targeting RNA, or guide RNA, leads the entire machinery to a particular region of DNA, where the Cas9 nuclease, acting like scissors, cleaves the DNA. When the cell's natural repair pathways kick in, the gene of interest is effectively edited.

Perturb-seq is an evolution of the CRISPR-Cas9 technology that uses its gene targeting capability to selectively activate or inactivate gene expression of genes of interest. This is combined with single-cell whole transcriptome gene expression analysis to interrogate the effects of the CRISPR-Cas9-mediated gene activation or inactivation on the whole transcriptome.

Now, Illumina scientists have developed a high-throughput method that allows researchers to interrogate the whole transcriptome of individual cells following gene perturbation by CRISPR-Cas9. These methods are being presented at the Advances in Genome Biology and Technology (AGBT) meeting in February 2025, in Florida.[2]

"Illumina's method is particularly important because it allows researchers to efficiently investigate the interactions between genotype and phenotype following CRISPR-Cas9-mediated gene expression perturbation, enabling them to understand specific pathways at an unprecedented scale," says Kristina Fontanez, senior director of product development at Illumina. She is also a cofounder of Fluent BioSciences, a Massachusetts-based biotechnology company focused on high-throughput tools for single-cell analysis, that was acquired last year by Illumina.

Previously, to understand the impact that a single-gene knockout had on phenotype, researchers had to invest years in developing mouse models and conducting follow-up studies. Now, with Illumina's scalable CRISPR screening methods, "you [have] the statistical power to

1. nature.com/articles/s41392-023-01309-7
2. agbt.org/home/home/general-meeting

evaluate the impact of knocking out every gene, one at a time, on a cell-by-cell basis," explains Robert Meltzer, associate principal scientist at Illumina and a fellow cofounder of Fluent BioSciences. "You can do that in the context of different tissue types, species, and organisms. This is the kind of technology that researchers have been dreaming about for five to 10 years, and now you have the sample preparation, sequencing, and analysis capacity to make that a routine operation."

Researchers have long struggled to develop scalable methods for analyzing the genome and transcriptome of combinatorial CRISPR screens. According to Meltzer, traditional methods are limited in capacity to about 10,000 cell batches. That is sufficient to evaluate hundreds of individual CRISPR guides, or perturbations, in a single experiment. "Illumina's upcoming 1 million cell reaction scale will allow for routine processing of 10,000 guide RNA screens with outstanding statistical power," he explains. "In combination with Illumina's high-capacity NovaSeq X sequencer and reagents, and DRAGEN accelerated analysis, genome-wide CRISPR screens become not only feasible, but practical and routine."

The success of the massive scale-up relies on polymers called "particle-templated instant partitions," or PIPs. PIPs were initially developed by physicist Adam Abate at the University of California, San Francisco. They are hydrogel particles that help emulsify a mixture and spontaneously separate cells during vortexing into hundreds of thousands, or even millions, of uniform droplets, which can then be subjected to transcriptome sequencing. Fluent BioSciences developed PIPs into a massively scalable single-cell technology for gene expression profiling with a next-generation-sequencing readout. The foundational Fluent PIPseq technology is now known as Illumina Single Cell 3' RNA Prep.[3] "It really changes the accessibility of single-cell technology," says Fontanez.

To get single-cell resolution of CRISPR-Cas9-perturbed cells, Fontanez and her colleagues modified the PIPs to recognize the CRISPR guide RNA backbone as well as mRNA. This way, when a single cell is captured inside a single droplet, the PIP enables identification of the particular guide RNA that is present inside the cell along with the cellular transcriptome response to the CRISPR-Cas9-mediated gene perturbation.

PIPs helped solve the issue of preparing high-throughput samples for single-cell analysis, and the Illumina NovaSeq X efficiently analyzes the CRISPR-Cas9-perturbed transcriptomes of those cells. DRAGEN, Illumina's analysis tool, is uniquely powered to analyze those datasets, a process that can be very computationally intensive.

Fontanez and Meltzer are enthusiastic about scaling this assay. But in the near term, they're keen on getting this technique to others. Meltzer says, "The applications that we could develop are myriad, but the goal is to put this into customers' hands as rapidly as possible." ◆

3. illumina.com/products/by-type/sequencing-kits/library-prep-kits/single-cell-rna-prep.html