

Speeding up gene discovery

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Since the completion of the Human Genome Project, which identified nearly 20,000 protein-coding genes, scientists have been trying to decipher the roles of those genes. A new approach developed at MIT, the Broad Institute, and the Whitehead Institute should speed up the process by allowing researchers to study the entire genome at once.

The new system, known as CRISPR, allows researchers to permanently and selectively delete [genes](#) from a cell's DNA. In two new papers, the researchers showed that they could study all the genes in the genome by deleting a different gene in each of a huge population of cells, then observing which cells proliferated under different conditions.

"With this work, it is now possible to conduct systematic genetic screens in mammalian cells. This will greatly aid efforts to understand the function of both protein-coding genes as well as noncoding genetic elements," says David Sabatini, a member of the Whitehead Institute, MIT professor of biology, and senior author of one of the papers, both of which appear in this week's online edition of *Science*.

Using this approach, the researchers were able to identify genes that allow [melanoma cells](#) to proliferate, as well as genes that confer resistance to certain chemotherapy drugs. Such studies could help scientists develop targeted cancer treatments by revealing the genes that [cancer cells](#) depend on to survive.

Feng Zhang, the W.M. Keck Assistant Professor in Biomedical Engineering and senior author of the other *Science* paper, developed the

CRISPR system by exploiting a naturally occurring bacterial protein that recognizes and snips viral DNA. This protein, known as Cas9, is recruited by short RNA molecules called guides, which bind to the DNA to be cut. This DNA-editing complex offers very precise control over which genes are disrupted, by simply changing the sequence of the RNA guide.

"One of the things we've realized is that you can easily reprogram these enzymes with a short nucleic-acid chain. This paper takes advantage of that and shows that you can scale that to large numbers and really sample across the whole genome," says Zhang, who is also a member of MIT's McGovern Institute for Brain Research and the Broad Institute.

Genome-wide screens

For their new paper, Zhang and colleagues created a library of about 65,000 guide RNA strands that target nearly every known gene. They delivered genes for these guides, along with genes for the CRISPR machinery, to human cells. Each cell took up one of the guides, and the gene targeted by that guide was deleted. If the gene lost was necessary for survival, the cell died.

"This is the first work that really introduces so many mutations in a controlled fashion, which really opens a lot of possibilities in functional genomics," says Ophir Shalem, a Broad Institute postdoc and one of the lead authors of the Zhang paper, along with Broad Institute postdoc Neville Sanjana.

This approach enabled the researchers to identify genes essential to the survival of two populations of cells: cancer cells and [pluripotent stem cells](#). The researchers also identified genes necessary for melanoma cells to survive treatment with the chemotherapy drug vemurafenib.

In the other paper, led by Sabatini and Eric Lander, the director of the Broad Institute and an MIT professor of biology, the research team targeted a smaller set of about 7,000 genes, but they designed more RNA guide sequences for each gene. The researchers expected that each sequence would block its target gene equally well, but they found that cells with different guides for the same gene had varying survival rates.

"That suggested that there were intrinsic differences between guide RNA sequences that led to differences in their efficiency at cleaving the genomic DNA," says Tim Wang, an MIT graduate student in biology and lead author of the paper.

From that data, the researchers deduced some rules that appear to govern the efficiency of the CRISPR-Cas9 system. They then used those rules to create an algorithm that can predict the most successful sequences to target a given gene.

"These papers together demonstrate the extraordinary power and versatility of the CRISPR-Cas9 system as a tool for genomewide discovery of the mechanisms underlying mammalian biology," Lander says. "And we are just at the beginning: We're still uncovering the capabilities of this system and its many applications."

Efficient alternative

The researchers say that the CRISPR approach could offer a more efficient and reliable alternative to RNA interference (RNAi), which is currently the most widely used method for studying gene functions. RNAi works by delivering short RNA strands known as shRNA that destroy messenger RNA (mRNA), which carries DNA's instructions to the rest of the cell.

The drawback to RNAi is that it targets mRNA and not DNA, so it is

impossible to get 100 percent elimination of the gene. "CRISPR can completely deplete a given protein in a cell, whereas shRNA will reduce the levels but it will never reach complete depletion," Zhang says.

In future studies, the researchers plan to conduct genomewide screens of cells that have become cancerous through the loss of [tumor suppressor genes](#) such as BRCA1. If scientists can discover which genes are necessary for those cells to thrive, they may be able to develop drugs that are highly cancer-specific, Wang says.

This strategy could also be used to help find drugs that counterattack tumor cells that have developed resistance to existing chemotherapy drugs, by identifying genes that those [cells](#) rely on for survival.

The researchers also hope to use the CRISPR system to study the function of the vast majority of the genome that does not code for proteins. "Only 2 percent of the genome is coding. That's what these two studies have focused on, that 2 percent, but really there's that other 98 percent which for a long time has been like dark matter," Sanjana says.

More information: "Genome-Scale CRISPR-Cas9 Knockout Screening in Human Cells" *Science*, 2013.

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