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Genome-editing 'toolbox' targets multiple genes at once

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A Yale research team has designed a system to modify, or edit, multiple genes in the genome simultaneously, while also minimizing unintended effects. The gene-editing "toolbox" provides a user-friendly solution that scientists can apply to research on cancer and other disciplines, the researchers said.

The study was published on July 26 in *Nucleic Acids Research*.

With modern genetic engineering techniques, such as the recently developed CRISPR technologies, researchers can edit genes in experiments. This rapidly evolving field allows researchers to study important disease-related genes and may ultimately allow them to treat genetic diseases by making edits in specific sites of the human genome. However, progress has been hampered by several challenges, including the editing of unintended sites—referred to as off-target effects.

Senior study author Qin Yan and his co-authors at Yale School of Medicine tackled these challenges in multiple ways. In previous systems, the genome-editing machinery was constantly turned on, leading to more off-target effects. To avoid these effects, the team first developed a strategy to make the machinery "inducible"—able to be turned on and off. They used a drug to turn the machinery on just long enough to edit the genes. This approach allowed them to activate the machinery as needed and for a limited amount of time, reducing off-target effects, they said.

Their gene-editing system also contained a fluorescent marker so the researchers could track whether the editing enzyme was turned on or off in cells, noted Yan, who is an associate professor of pathology and member of Yale Cancer Center.

The next step was to make the process of gene editing less complex and time-consuming than previous systems. To target multiple genes simultaneously, the research team developed a simple strategy to put multiple targeting sequences in one step. Each targeting sequence allows the editing machinery to recognize one gene.

With the combined gene-editing strategies—the toolbox—the researchers focused on a group of three cancer-related genes. In experiments using both cultured cells and animal models, they simultaneously deleted all three genes.

"This toolbox can be used for inducible and multiple-gene targeting," said Yan. "We streamlined what used to be a very complicated process, making it more efficient and much simpler to edit several genes at once—major advantages that other systems do not have."

The design of the system also allowed the team to conduct their work in a condensed period of time. "This toolbox will shorten our experiments significantly, from a month to a week. It's very efficient," said Jian Cao, associate research scientist of pathology and first author of the study.

In addition to its application as a stand-alone system, the toolbox could be combined with other techniques to improve gene editing in biomedical research and other fields, according to the researchers.

"The overall goal is an efficient platform, and to overcome some of the problems current systems have," said Yan.

More information: Jian Cao et al, An easy and efficient inducible CRISPR/Cas9 platform with improved specificity for multiple gene targeting, *Nucleic Acids Research* (2016). DOI: 10.1093/nar/gkw660