A guide to CRISPR gene activation

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The CRISPR-Cas9 system has come to be known as the quintessential tool that allows researchers to edit the DNA sequences of many organisms and cell types. However, scientists are also increasingly recognizing that it can be used to activate the expression of genes. To that end, they have built a number of synthetic gene activating Cas9 proteins to study gene functions or to compensate for insufficient gene expression in potential therapeutic approaches.

"The possibility to selectively activate genes using various engineered variants of the CRISPR-Cas9 system left many researchers questioning which of the available synthetic activating Cas9 proteins to use for their purposes. The main challenge was that all had been uniquely designed and tested in different settings; there was no side-by-side comparison of their relative potentials," said George Church, Ph.D., who is Core Faculty Member at the Wyss Institute for Biologically Inspired Engineering at Harvard University, leader of its Synthetic Biology Platform, and Professor of Genetics at Harvard Medical School. "We wanted to provide that side-by-side comparison to the biomedical research community."

In a study published on 23 May in *Nature Methods*, the Wyss Institute team reports how it rigorously compared and ranked the most commonly used artificial Cas9 activators in different cell types from organisms including humans, mice and flies. The findings provide a valuable guide to researchers, allowing them to streamline their endeavors.

The team also included Wyss Core Faculty Member James Collins,

Ph.D., who also is the Termeer Professor of Medical Engineering & Science and Professor of Biological Engineering at the Massachusetts Institute of Technology (MIT)'s Department of Biological Engineering and Norbert Perrimon, Ph.D., a Professor of Genetics at Harvard Medical School.

Gene activating Cas9 proteins are fused to variable domains borrowed from proteins with well-known gene activation potentials and engineered so that the DNA editing ability has been destroyed. In some cases, the second component of the CRISPR-Cas9 system, the guide RNA that targets the complex to specific DNA sequences, also has been engineered to bind gene-activating factors.

"We first surveyed seven advanced Cas9 activators, comparing them to each other and the original Cas9 activator that served to provide proof-of-concept for the gene activation potential of CRISPR-Cas9. Three of them, provided much higher gene activation than the other candidates while maintaining high specificities toward their target genes," said Marcelle Tuttle, Research Fellow at the Wyss and a co-lead author of the study.

The team went on to show that the three top candidates were comparable in driving the highest level of gene expression in cells from humans, mice and fruit flies, irrespective of their tissue and developmental origins. The researchers also pinpointed ways to further maximize gene activation employing the three leading candidates.

"In some cases, maximum possible activation of a target gene is necessary to achieve a cellular or therapeutic effect. We managed to cooperatively enhance expression of specific genes when we targeted them with three copies of a top performing activator using three different guide RNAs," said Alejandro Chavez, Ph.D., a Postdoctoral Fellow and the study's co-first author.

"The ease of use of CRISPR-Cas9 offers enormous potential for development of genome therapeutics. This study provides valuable new design criteria that will help enable synthetic biologists and bioengineers to develop more effective targeted genome engineering technologies in the future," said Wyss Institute Founding Director Donald Ingber, M.D., Ph.D., who is the Judah Folkman Professor of Vascular Biology at Harvard Medical School and the Vascular Biology Program at Boston Children's Hospital, and also Professor of Bioengineering at the Harvard John A. Paulson School of Engineering and Applied Sciences.

More information: Comparison of Cas9 activators in multiple species, *Nature Methods*, DOI: 10.1038/nmeth.3871

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