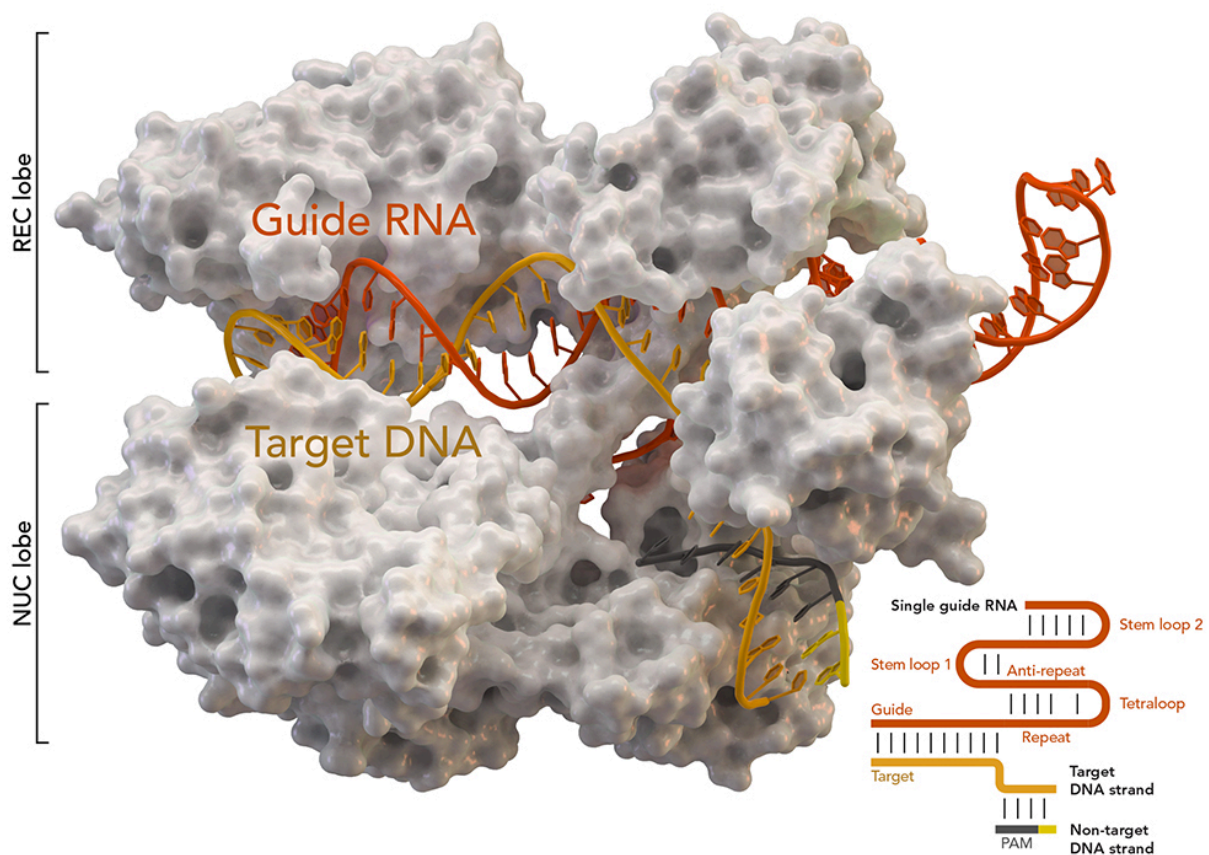


New method to detect off-target effects of CRISPR

April 19 2019, by Julie Langelier



CRISPR-associated protein Cas9 (white) from *Staphylococcus aureus* based on Protein Database ID 5AXW. Credit: Thomas Splettstoesser (Wikipedia, CC BY-SA 4.0)

Since the CRISPR genome editing technology was invented in 2012, it has shown great promise to treat a number of intractable diseases. However, scientists have struggled to identify potential off-target effects in therapeutically relevant cell types, which remains the main barrier to moving therapies to the clinic. Now, a group of scientists at the Gladstone Institutes and the Innovative Genomics Institute (IGI), with collaborators at AstraZeneca, have developed a reliable method to do just that.

CRISPR edits a person's genome by cutting the DNA at a specific location. The challenge is to ensure the tool doesn't also make cuts elsewhere along the DNA—damage referred to as "off-target effects," which could have unforeseen consequences.

In a study to be published tomorrow in the journal *Science*, the two first authors, Beeke Wienert and Stacia Wyman, found a new way to approach the problem.

"When CRISPR makes a cut, the DNA is broken," says Wienert, Ph.D., who began the work in Jacob E. Corn's IGI laboratory and who is now a postdoctoral scholar in Bruce R. Conklin's laboratory at Gladstone. "So, in order to survive, the cell recruits many different DNA repair factors to that particular site in the genome to fix the break and join the cut ends back together. We thought that if we could find the locations of these DNA repair factors, we could identify the sites that have been cut by CRISPR."

To test their idea, the researchers studied a panel of different DNA repair factors. They found that one of them, called MRE11, is one of the first responders to the site of the cut. Using MRE11, the scientists developed a new technique, named DISCOVER-Seq, that can identify the exact sites in the genome where a cut has been made by CRISPR.

"The human genome is extremely large—if you printed the entire DNA sequence, you would end up with a novel as tall as a 16-story building," explains Conklin, MD, senior investigator at Gladstone and deputy director at IGI. "When we want to cut DNA with CRISPR, it's like we're trying to remove one specific word on a particular page in that novel."

"You can think of the DNA repair factors as different types of bookmarks added to the book," Conklin adds. "While some may bookmark an entire chapter, MRE11 is a bookmark that drills down to the exact letter that has been changed."

Different methods currently exist to detect CRISPR off-target effects. However, they come with limitations that range from producing [false positive results](#) to killing the cells they're examining. In addition, the most common method used to date is currently limited to being used in cultured cells in the laboratory, excluding its use in patient-derived stem cells or animal tissue.

"Because our method relies on the cell's natural repair process to identify cuts, it has proven to be much less invasive and much more reliable," says Corn, Ph.D., who now runs a laboratory at ETH Zurich. "We were able to test our new DISCOVER-Seq method in induced pluripotent [stem cells](#), patient cells, and mice, and our findings indicate that this method could potentially be used in any system, rather than just in the lab."

The DISCOVER-Seq method, by being applied to new cell types and systems, has also revealed new insights into the mechanisms used by CRISPR to edit the genome, which will lead to a better understanding of the biology of how this tool works.

"The new method greatly simplifies the process of identifying off-target effects while also increasing the accuracy of the results," says Conklin,

who is also a professor of medical genetics and molecular pharmacology at UC San Francisco (UCSF). "This could allow us to better predict how [genome](#) editing would work in a clinical setting. As a result, it represents an essential step in improving pre-clinical studies and bringing CRISPR-based therapies closer to the patients in need."

The paper "Unbiased detection of CRISPR off-targets in vivo 1 using DISCOVER-Seq" will be published by the journal *Science* on April 19, 2019.

More information: Unbiased detection of CRISPR off-targets in vivo using DISCOVER-Seq, *Science* 19 Apr 2019: Vol. 364, Issue 6437, pp. 286-289 , [DOI: 10.1126/science.aav9023](https://doi.org/10.1126/science.aav9023) , science.sciencemag.org/content/364/6437/286

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