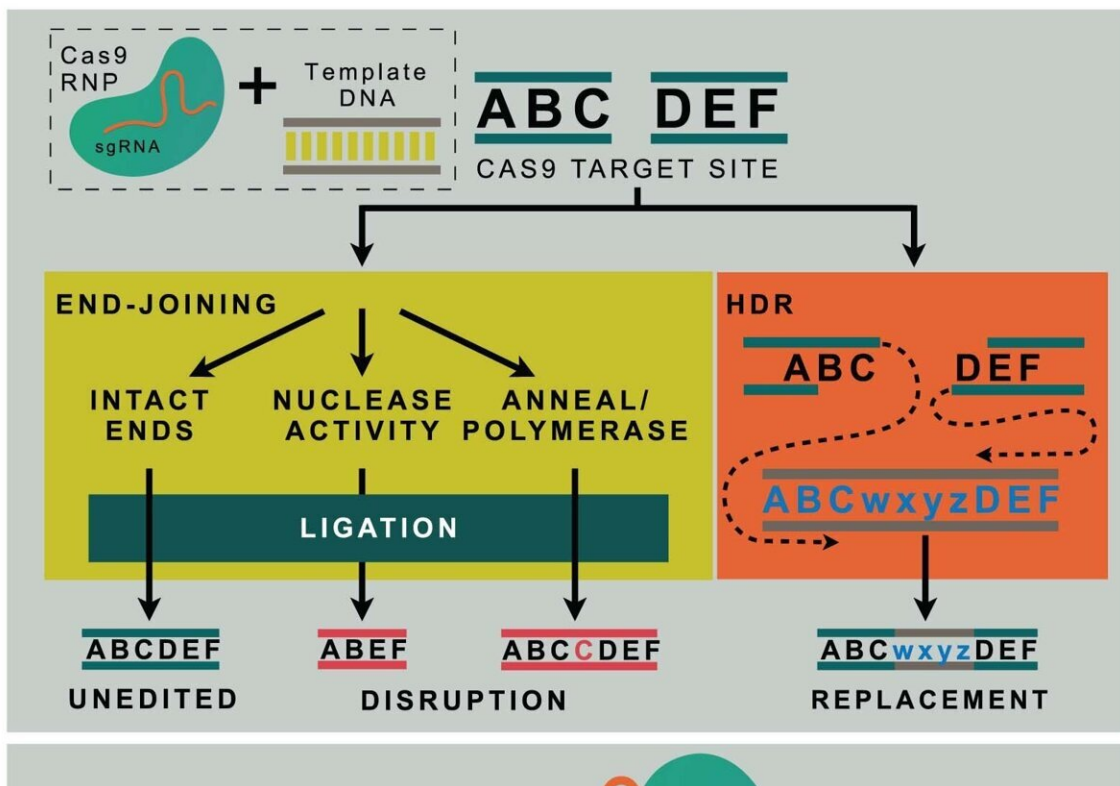


# Researchers discover a way to improve nonviral gene editing as well as a new type of DNA repair

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Modification of HDRTs with interstrand crosslinks increases HR during gene editing. (a) Top panel: Cas9 RNPs introduce a double strand DNA break (DSB) at a targeted region in the genome, which can be repaired by error prone end joining (EJ) processes that rejoin the ends of the break, or homology-directed repair (HDR) processes that resolve DSBs using sequence encoded in a separate template molecule. Bottom panel: HDR gene editing applications can be

approximated using marker-based assays as diagrammed. These editing events are initiated by electroporation of Cas9, sgRNA, and HDRT into human cells, and monitored by flow cytometry or high throughput sequencing. b)

Incorporation frequency of a pSFFV-GFP construct into the HBB locus of K562 cells using plasmid DNA treated with the indicated amount of cisplatin, data displayed as the mean  $\pm$  SD of  $n = 3$  biological replicates. (c) Transcription is inhibited from xHDRTs. Expression of dox-inducible mCherry presented both as mean fluorescence intensity (MFI) (left) and percent (right) of cells expressing mCherry encoded by uncrosslinked or xHDRT plasmid DNA. Data displayed as the mean  $\pm$  SD of  $n = 3$  biological replicates. Credit: *Nature Biotechnology* (2023). DOI: 10.1038/s41587-022-01654-y

Gene editing is a powerful method for both research and therapy. Since the advent of the Nobel Prize-winning CRISPR/Cas9 technology, a quick and accurate tool for genome editing discovered in 2012, scientists have been working to explore its capabilities and boost its performance.

Researchers in UC Santa Barbara biologist Chris Richardson's lab have added to that growing toolbox, with a method that increases the efficiency of CRISPR/Cas9 editing without the use of viral material to deliver the genetic template used to edit the target genetic sequence. According to their new paper published in the journal *Nature Biotechnology*, their method stimulates homology-directed repair (a step in the gene editing process) by approximately threefold "without increasing mutation frequencies or altering end-joining repair outcomes."

"We've found a chemical modification that improves non-viral gene editing and also discovered an intriguing new type of DNA repair," Richardson said.

## **Find, cut and paste**

The CRISPR/Cas9 method works by capitalizing on a defense technique employed by bacteria against viral attackers. To do this, the bacteria snip a piece of the invading virus's genetic material, and incorporate it into their own in order to recognize it later. Should the bacteria get reinfected, they can target the now-familiar genetic sequences for destruction.

In gene editing, this process uses the enzyme Cas9 as molecular "scissors" to snip sequences it recognizes, guided by the CRISPR system. This cut is also an opportunity to replace the severed genes with similar (homologous) but improved ones, utilizing the cell's natural repair mechanisms. If successful, the cell should have modified expressions and functions thereafter.

To deliver the repair template DNA to the nucleus of the cell where its genetic material lives, oftentimes viruses are used. While they are effective, the researchers say, viral workflows "are expensive, difficult to scale and potentially toxic to cells."

Nonviral templates are potentially less expensive and more scalable, although researchers still must overcome efficiency and toxicity barriers. In their study, the Richardson Lab found that introducing interstrand crosslinks into the workflow increased homology-directed repair dramatically.

"Every workflow that we have put this approach into has worked better by roughly threefold," Richardson said.

Interstrand crosslinks are lesions that keep the double strands of a DNA helix tethered to each other, making them unable to replicate. Cancer chemotherapies use this mechanism to interrupt tumor growth and kill cancer cells. Added to a homology-directed repair template, however, these crosslinks were found to stimulate the cell's natural repair

mechanisms and increase the likelihood of editing success.

"Basically, what we've done is taken this template DNA and damaged it," Richardson said. "We've in fact damaged it in the most severe way I can think of. And the cell doesn't say, 'Hey this is junk; let me throw it away.' What the cell actually says is, 'Hey this looks great; let me stick it into my genome.'" The result is a highly efficient and minimally error-prone nonviral system of gene editing.

Their discovery, like many breakthroughs in science, was actually something of a happy accident. While working to purify proteins to study DNA repair, graduate student researcher and lead author Hannah Ghasemi noted unanticipated changes to the outcomes of their experiments.

"We were introducing these chemical modifications to the DNA templates in order to be able to pull them out of the cells and see what proteins were bound to them, and I was just checking to see if this modification had somehow affected the editing in any capacity," she said. "I was expecting to either see no change or that it actually might have negatively affected the editing."

What she found instead was a positive effect, up to three times the editing activity of the uncrosslinked controls. Furthermore, the team found that even with the increase in edits—and therefore the chances for errors—there was no increase in mutation frequency. They are still investigating the specific mechanisms leading to this result, but they have ideas.

"What we think happens is that the cell detects and tries to repair the damaged DNA that we've added this crosslink to," Richardson said. "And in doing so, it delays the cell past a checkpoint where it would normally stop this recombination process. And so by prolonging the

amount of time that it takes the cell to do this [recombination](#), it makes it more likely that the edits will go to completion." Studying this new process could also lead to a better understanding about how cells detect editing reagents and how they "decide" to accept them or not, he said.

This method will find the most use in ex-vivo gene editing applications, according to the team; that is, in the realm of disease research and preclinical work.

"We can more effectively knock down genes and insert things into genomes to study systems outside of the human body in a lab setting," Ghasemi said. This development allows them to more efficiently build disease models and test hypotheses about how diseases work, which could lead to better clinical and therapeutic approaches.

**More information:** Hannah I. Ghasemi et al, Interstrand crosslinking of homologous repair template DNA enhances gene editing in human cells, *Nature Biotechnology* (2023). [DOI: 10.1038/s41587-022-01654-y](https://doi.org/10.1038/s41587-022-01654-y)

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