

'Soft' CRISPR may offer a new fix for genetic defects

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Restorative gene editing using sequences from the counterpart chromosome: The standard CRISPR enzyme Cas9 offers the ability to make repairs but also potentially results in unintended mutations (mutagenic events) at the targeted site and possibly elsewhere in the genome (left). In contrast, the nickase enzyme results in more efficient gene correction and no mutagenic events (right). Credit: Guichard/Bier

Curing debilitating genetic diseases is one of the great challenges of modern medicine. During the past decade, development of CRISPR technologies and advancements in genetics research brought new hope for patients and their families, although the safety of these new methods is still of significant concern.

Publishing July 1 in the journal *Science Advances*, a team of biologists at the University of California San Diego that includes postdoctoral scholar Sitara Roy, specialist Annabel Guichard and Professor Ethan Bier describes a new, safer approach that may correct [genetic defects](#) in the future. Their strategy, which makes use of natural DNA repair machinery, provides a foundation for novel gene therapy strategies with the potential to cure a large spectrum of [genetic diseases](#).

In many cases, those suffering from genetic disorders carry distinct mutations in the two copies of genes inherited from their parents. This means that often, a mutation on one chromosome will have a functional sequence counterpart on the other chromosome. The researchers employed CRISPR genetic editing tools to exploit this fact.

"The healthy variant can be used by the cell's repair machinery to correct the defective mutation after cutting the mutant DNA," said Guichard, the senior author of the study, "Remarkably, this can be achieved even more efficiently by a simple harmless nick."

Working in [fruit flies](#), the researchers designed mutants permitting visualization of such "homologous chromosome-templated repair," or HDR, by the production of pigments in their eyes. Such mutants initially featured entirely white eyes. But when the same flies expressed CRISPR components (a guide RNA plus Cas9), they displayed large red patches across their eyes, a sign that the cell's DNA [repair machinery](#) had succeeded in reversing the mutation using the functional DNA from the other chromosome.

They then tested their new system with Cas9 variants known as "nickases" that targeted just one strand of DNA instead of both. Surprisingly, the authors found that such nicks also gave rise to high-level restoration of red eye color nearly on par with normal (non-mutated) healthy flies. They found a 50-70% repair success rate with the nickase compared with just 20-30% in dual-strand cutting Cas9, which also generates frequent mutations and targets other sites throughout the genome (so-called off-target mutations). "I could not believe how well the nickase worked—it was completely unanticipated," said Roy, the lead author of the study. The versatility of the new system could serve as a model for fixing [genetic mutations](#) in mammals, the researchers noted.

"We don't know yet how this process will translate to [human cells](#) and if we can apply it to any gene," said Guichard. "Some adjustment may be needed to obtain efficient HDR for disease-causing mutations carried by human chromosomes."

The new research extends the group's previous achievements in precision-editing with "[allelic-drives](#)," which expand on principles of gene-drives with a guide RNA that directs the CRISPR system to cut undesired variants of a gene and replace them with a preferred version of the gene.

A key feature of the team's research is that their nickase-based system causes far fewer on- and off-target mutations, as is known to happen with more traditional Cas9-based CRISPR edits. They also say a slow, continuous delivery of nickase components across several days may prove more beneficial than one-time deliveries.

"Another notable advantage of this approach is its simplicity," said Bier. "It relies on very few components and DNA nicks are 'soft,' unlike Cas9, which produces full DNA breaks often accompanied by mutations."

"If the frequency of such events could be increased either by promoting

interhomolog pairing or by optimizing nick-specific repair processes, such strategies could be harnessed to correct numerous dominant or trans-heterozygous disease-causing [mutations](#)," said Roy.

More information: Sitara Roy et al, Cas9/Nickase-induced allelic conversion by homologous chromosome-templated repair in *Drosophila* somatic cells, *Science Advances* (2022). DOI: [10.1126/sciadv.abo0721](https://doi.org/10.1126/sciadv.abo0721). www.science.org/doi/10.1126/sciadv.abo0721

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