New way to identify RNA variants could make CRISPR gene editing more effective

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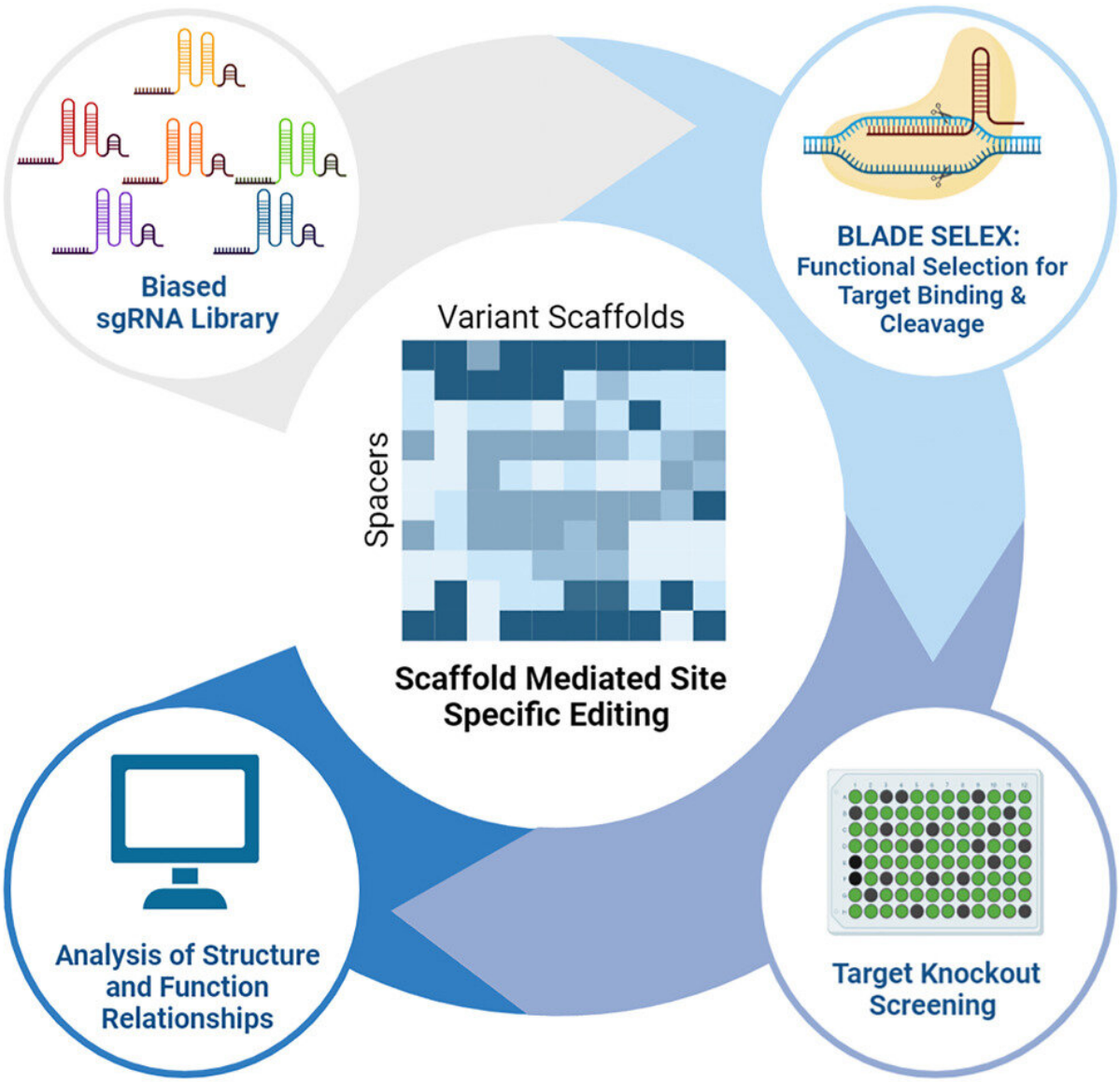

Credit: *Cell Chemical Biology* (2023). DOI: 10.1016/j.chembiol.2023.06.007

CRISPR gene editing is a breakthrough that has been used to treat diseases such as sickle cell anemia, leukemia and genetic disorders, but it has challenges that limit its broad utility.

Identifying the root of those issues led a research team at Duke Health to find an improved approach to gene editing that expands its functionality.

In work published online June 29 in the journal *Cell Chemical Biology*, the researchers lay out a new way to identify diverse CRISPR RNA variants that can specifically home in on challenging areas of DNA to target for editing. The new approach opens up more of the genome for editing, enabling the repair of mutations associated with more diseases.

"CRISPR is great, but there are a lot of places within the human genome that can't be edited well," said senior author Bruce Sullenger, Ph.D., the Joseph W. and Dorothy W. Beard Distinguished Professor of Experimental Surgery at Duke University School of Medicine. Sullenger is also a professor in the departments of Pharmacology and Cancer Biology, Neurosurgery, Cell Biology and Biomedical Engineering.

"This work started from ground zero to understand and address that issue using large libraries of different CRISPR RNAs as well as informatics to see if we could get these editing tools to more places in the genome so that they can be edited and repaired as needed," Sullenger said.

Sullenger and colleagues—notably first author Korie Bush, Ph.D., who launched the study as a graduate student in Sullenger's lab—sought to improve the process CRISPR relies on to guide RNA to the right location on DNA and facilitate the deletion or repair in that region.

More often than not, the guide, which is an RNA molecule, presents a problem—it may not fold exactly right or it's otherwise corrupted, so the editing or deletion process cannot occur. Typically when that happens, the guide RNA must be swapped out for a new one and the genome cannot be targeted in the right spot.

The Duke team instead found a way to salvage a dysfunctional guide RNA, which actually has two components that have to work well together: an RNA sequence that recognizes the DNA target site and a scaffolding sequence that holds the enzyme in place to cleave the DNA at the right juncture.

They found multiple RNA sequences that revived the integrity of the scaffolding, demonstrating that CRISPR gene editing technology is much more adaptable than previously believed.

"We found lots of ways to change the system that yield combinations with enhanced editing efficiencies at various target sites," Bush said. "The process is more malleable than we thought—we made hundreds of thousands of guide RNAs. If you need two pieces for the process to work well and you have so many more options to choose from, you can find a combination that works best."

Sullenger said the finding should lead to safer and more effective ways of applying CRISPR technology as a therapy.

"This is like an improvement in word processing that enables you to easily find and edit any error in the text, rather than only being able to edit certain portions of a text effectively," Sullenger said. "Especially when coupled with informatics, it should lead to an acceleration of breakthroughs for treating diseases."

More information: Korie Bush et al, Utilizing directed evolution to

interrogate and optimize CRISPR/Cas guide RNA scaffolds, *Cell Chemical Biology* (2023). DOI: 10.1016/j.chembiol.2023.06.007