Potential new gene editing tools uncovered

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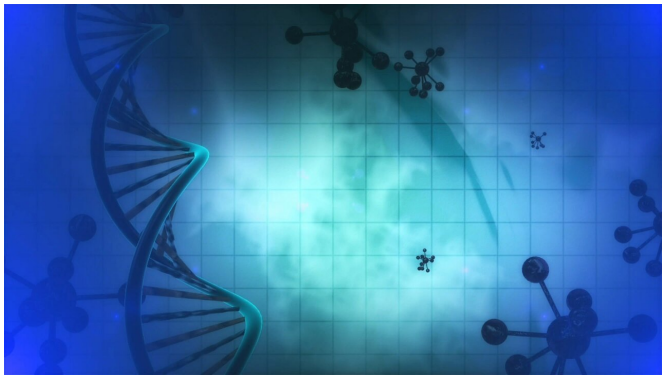

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Few developments have rocked the biotechnology world or generated as much buzz as the discovery of CRISPR-Cas systems, a breakthrough in gene editing recognized in 2020 with a Nobel Prize. But these systems that naturally occur in bacteria are limited because they can make only small tweaks to genes. In recent years, scientists discovered a different system in bacteria that might lead to even more powerful methods for gene editing, given its unique ability to insert genes or whole sections of DNA in a genome.

New research from The University of Texas at Austin dramatically expands the number of naturally occurring versions of this system, giving researchers a wealth of potential new tools for large-scale gene editing.

Other scientists had identified clusters of genes that use CRISPR to insert themselves into different places in an organism's genome, dubbed CRISPR-associated transposons (CASTs). Earlier work has shown they can be used to add an entire gene or large DNA sequence to the genome, at least for bacteria.

Now a team led by Ilya Finkelstein and Claus Wilke at UT Austin have expanded the number of likely CASTs from about a dozen to nearly 1,500. They published their results this week in the journal *Proceedings of the National Academy of Sciences*.

"With CASTs, we could potentially insert lots of genes, called 'gene cassettes,' encoding multiple complicated functions," said Finkelstein, associate professor of molecular biosciences, who conceived and headed the research. Among other things, this opens up the possibility of treating complex diseases associated with more than one gene.

CRISPR researcher and Nobel laureate Jennifer Doudna has predicted CASTs will be a critical element in expanding genetic engineers' toolkit, making it possible to introduce "any change, at any genetic location, in any organism" within the decade, according to Genetic Engineering and Biotechnology News.

Using the Stampede2 supercomputer at the Texas Advanced Computing Center (TACC), the team combed through the world's largest database of genome fragments from microbes that have not yet been cultured in the lab or fully sequenced.

"Without the resources of TACC, this would have been impossible," said Wilke, professor and chair of the Department of Integrative Biology, who led the data-engineering part of the project.

He estimates that if the search was run on a powerful desktop computer, it would have taken years. Instead, with one of the university's supercomputers, the final analysis was completed within a few weeks. Three graduate students—James Rybarski, Kuang Hu and Alexis Hill—worked full time on various aspects of the project for nearly two years.

"The term for this is bioprospecting," Finkelstein said. "It was like sifting through a lot of silt and junk to find the occasional gold nugget."

The UT Austin team found 1,476 new putative CASTs, including three new families, doubling the number of known families. They have already

exerpimentally verified several of these and plan to continue testing more. Ultimately, Finkelstein predicts most will turn out to be true CASTs.

"If you have just a handful [of CASTs], it's unlikely that you have the best ones in existence," Wilke said. "By having more than a thousand, we can start to find out which ones are easiest to work with or most efficient or accurate. Hopefully there are new gene-editing systems that can do things better than the systems we had beforehand."

In the short term, Finkelstein said many of these new systems should be adaptable to genetically engineering bacteria. The long-term challenge, Finkelstein said, is to "domesticate" the systems to work in our cells.

"The holy grail is to get this working in mammalian cells," Finkelstein said.

More information: James R. Rybarski et al, Metagenomic discovery of CRISPR-associated transposons, *Proceedings of the National Academy of Sciences* (2021). DOI: 10.1073/pnas.2112279118

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