Genome editing: Pressing the 'delete' button on DNA

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Deletion of genomic DNA by paired CRISPR. Cas9 proteins (scissors) are guided to their target sites by single guide RNAs (sgRNAs, orange ribbons). The target region in between is removed. CRISPETa software enables researcher to design such deletion experiments quickly and conveniently. Credit: Pulido-Quetglas et al, CC BY

Until recently, genomics was a "read-only" science, but scientists have

developed a tool for quick and easy deletion of DNA in living cells. This software, published in *PLOS Computational Biology*, will boost efforts to understand the vast regions of non-coding DNA, or "Dark Matter", in our DNA and may lead to discovery of new disease-causing genes and potential new drugs.

CRISPR-Cas9 is a revolutionary technique for editing genomes and until recently, most studies employing it were aimed at silencing protein-coding genes, the best-studied part of our genome. However our genome consists of 99% of DNA that does not encode any protein. Often described as the "Dark Matter" of the genome, this "non-coding DNA" is recognised to be crucially important for understanding all aspects of human biology, including disease and evolution.

The Johnson lab recently created a tool based on CRISPR-Cas9, called "DECKO", which can be used to delete any desired piece of non-coding DNA. The unique advantage of DECKO is that it uses two individual sgRNAs, acting like two "molecular scissors" that snip out a piece of DNA. The approach was widely adopted, but as no software was available for designing the pairs of sgRNAs that are required, designing deletion experiments was time-consuming.

In response to this, the researchers in this study led by Carlos Pulido, created a software pipeline called CRISPETa, a flexible solution for designing CRISPR deletion experiments. The user tells CRISPETa what region they wish to delete, and the software returns a set of optimised pairs of sgRNAs that can directly be used by experimental researchers. One of the key features is that it can create designs at high scales, with future screening experiments in mind.

The researchers showed that CRISPETa designs efficiently delete their desired targets in human cells. Most importantly, in those regions that give rise to RNA molecules, the researchers showed that the RNA

molecules also carry the deletion.

"Ultimately, we expect that CRISPR deletion and other genome engineering tools to lead to a revolution in our ability to understand the genomic basis of disease, particularly in the 99% of DNA that does not encode proteins. Apart from being used as a basic research tool, CRISPR may even be used in the future as a powerful therapeutic to reverse disease-causing mutations," adds Rory Johnson.

CRISPETa is designed for use by non-experts so that it can be useful for scientific researchers, from even the most modest experimental laboratory. These users may, for example, delete a suspected functional region of non-coding DNA, and test the outcome on cellular or molecular activity. This software will also be potentially valuable for groups aiming to utilise CRISPR deletion for therapeutic purposes, by for example, deleting a region of non-coding DNA that is suspected to cause a disease state.

"We hope that this new software tool will allow the greatest possible number of researchers to harness the power of CRISPR deletion in their research," says Carlos Pulido, the student who wrote the CRISPETa software.

More information: Pulido-Quetglas C, Aparicio-Prat E, Arnan C, Polidori T, Hermoso T, Palumbo E, et al. (2017) Scalable Design of Paired CRISPR Guide RNAs for Genomic Deletion. *PLoS Comput Biol* 13(3): e1005341. DOI: 10.1371/journal.pcbi.1005341

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