When editing bacteria with CRISPR, less is more

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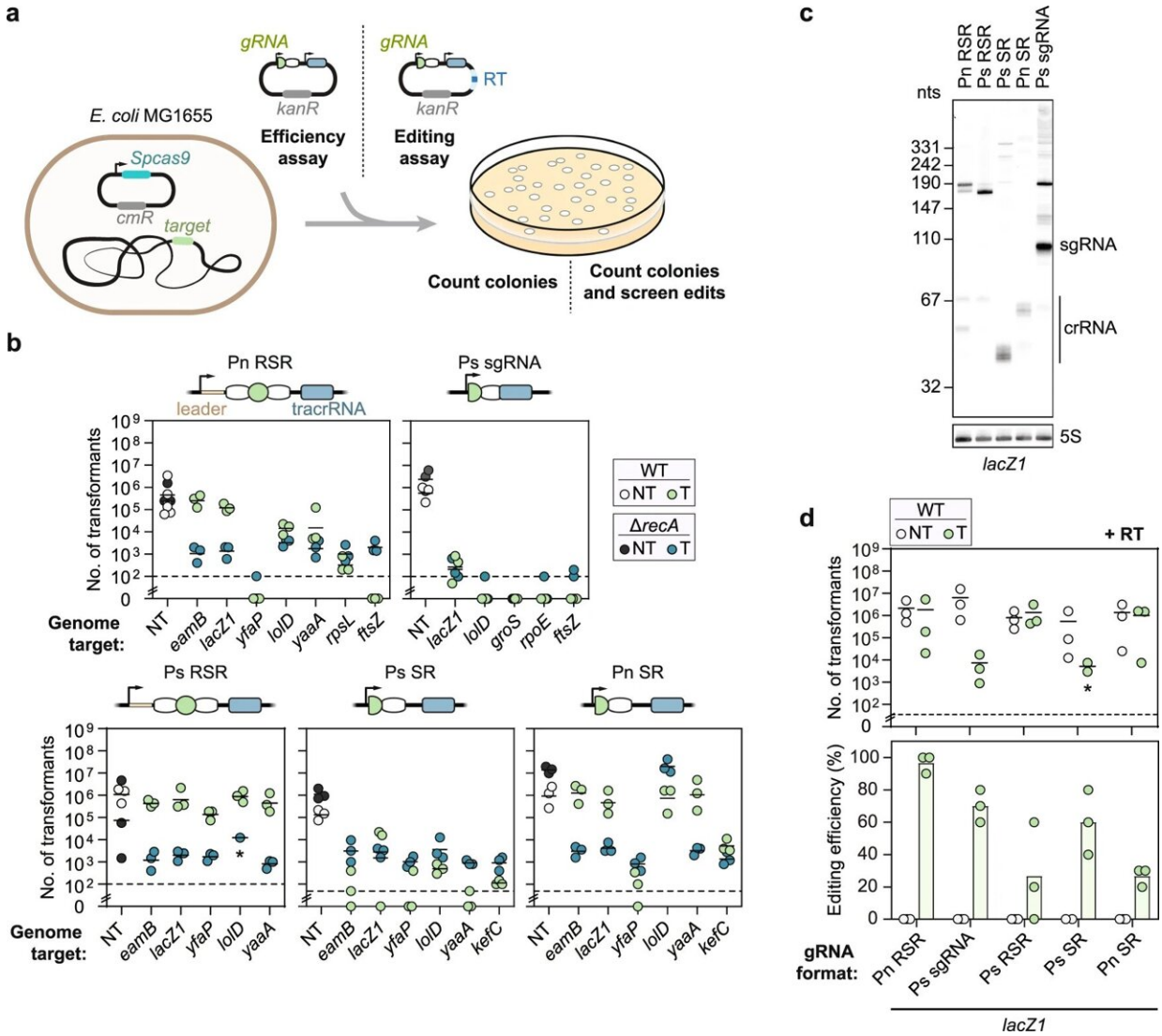

The outcome of chromosomal targeting in E. coli depends on gRNA format and abundance. **a** Schematic of the experimental setup for chromosomal targeting

and editing. **b** Genome targeting assay in *E. coli* and *E. coli* Δ*recA*. **c** Northern blot analysis of whole RNA isolated from *E. coli* Δ*lacI-Z* with pCas9 and pgRNA. A *lacZ1* spacer specific probe was used to probe the abundance of each RNA product. A 5 S probe was used as a control on the same gel and shown below the *lacZ1* probed gel. The approximate size of an sgRNA is indicated to the left of the Northern blot and the approximate size of mature crRNAs are indicated with a line. **d** Genome editing assay in *E. coli* targeting *lacZ1* to introduce AvrII restriction enzyme recognition site as a silent mutation. Individual dots for the transformations indicate a single biological replicate. * indicates that the transformants resulted in a lawn or uncountable colonies. Dashed lines indicate the limit of detection from plating. NT and T indicate targeting and non-targeting gRNAs, respectively. Individual dots for the editing efficiencies indicate the average of 3 colonies screened from one biological replicate for NT samples or 10 colonies screened from one biological replicate for targeting samples. Bars indicate the mean of the dots. The dashed line in (**b**, **d**) indicates the limit-of-detection. The limit-of-detection was calculated based on the volume of cells plated for each experiment. The mean number of transformants (indicated by a horizontal line) was not calculated for samples with biological replicates that fell below the limit of detection. WT indicates wild-type *E. coli* MG1655, Δ*recA* indicates *E. coli* MG1655 Δ*recA*, NT indicates non-targeting, T indicates targeting, and RT indicates repair template. Credit: *Nature Communications* (2023). DOI: 10.1038/s41467-023-36283-9

Systematically attenuating DNA targeting activity can achieve CRISPR-driven editing in bacteria, greatly boosting colony counts and even increasing the frequency of precise genome editing. This was shown in a study of the Helmholtz Institute Würzburg (HIRI) in collaboration with the Helmholtz Centre for Infection Research (HZI) in Braunschweig. The findings were published today in the journal *Nature Communications*.

The ability to genetically manipulate bacteria has been key to exploring the microbial world. Genome editing is crucial for the development of

new antibiotics and harnessing bacteria as miniature factories for the sustainable production of chemicals, materials, and therapeutics. Tools based on CRISPR "gene scissors" have proven instrumental in this regard, making it fast, easy, and reliable to create edits in different bacteria.

The general technology requires a CRISPR ribonucleic acid (crRNA) serving as a "guide RNA". It helps detect certain regions of a genome for targeted DNA cleavage. Proteins involved in homologous recombination—a natural process of exchanging genetic material between chromosomes—weave in designed "repair template" DNA afterwards to create an edited sequence of the strand.

Breaking the stumbling block

In the current study, researchers from the Würzburg Helmholtz Institute for RNA-based Infection Research (HIRI) in collaboration with the Helmholtz Centre for Infection Research (HZI) in Braunschweig address a central challenge to genome editing in bacteria.

"CRISPR-based genome editing has become a common molecular technology, but there is a notable stumbling block," says HIRI department head Chase Beisel, who led the study. "During exponential growth, bacteria initiate genome replication multiple times in one cell cycle to keep pace with cell division. By efficiently cutting DNA, CRISPR leads to the untimely death of the cell. Consequently, editing requires efficient recombination and high transformation efficiencies, which are unavailable in most bacterial strains, including those relevant to human disease and industrial biotechnology," Beisel explains.

A seemingly paradoxical concept

Daphne Collias, postdoc in Beisel's lab at the Helmholtz Institute Würzburg, is the first author of the study. Describing the background to the findings she says, "We found that attenuating the cutting activity of CRISPR would allow the cell to repair the cut DNA using the provided template for homologous recombination. As a result, we could drive homologous recombination and get many more surviving cells."

The researchers also developed a suite of approaches that could pull back activity, including using different formats for the guide RNA that directs cutting by the Cas9 protein, using versions of Cas9 that cut less efficiently, reducing guide RNA expression, introducing interfering structures onto the guide RNA, and mutating the sequence in the guide RNA used to find its DNA target.

"We called the modified guide RNAs 'attenuated guide RNAs' or atgRNAs, representing a flexible means of achieving CRISPR-driven editing," Collias reports. "Not every approach could drive editing, although we usually could find at least one for each editing setup."

Future impact

As a proof-of-principle, the Beisel lab teamed up with HZI department head Till Strowig and his lab to enhance editing in different strains of Klebsiella bacteria. Using a multidrug resistant strain, they leveraged editing to reverse resistance against the antibiotic ampicillin.

The new editing approach can advance basic research on bacteria involved in human health. Edited bacteria could also be used as therapeutic probiotics or as production hosts for therapeutics in the future.

More information: Daphne Collias et al, Systematically attenuating DNA targeting enables CRISPR-driven editing in bacteria, *Nature*

Communications (2023). DOI: 10.1038/s41467-023-36283-9

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