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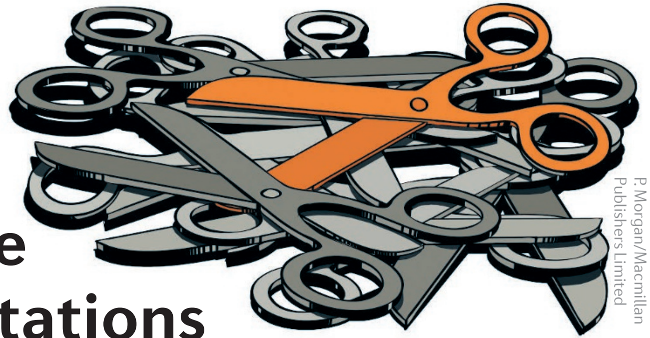
GENETIC ENGINEERING

CREATE-ing genome-wide designed mutations



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CRISPR–Cas genome engineering is progressively being applied for diverse high-throughput functional genomics screens. A new study reports CRISPR-enabled trackable genome engineering (CREATE) as a method for genome-scale generation of designed mutations in bacteria, with barcode-based monitoring of their phenotypic consequences.

High-throughput applications of CRISPR-Cas systems for functional genomics have generally followed one of two strategies. Targeting many genomic regions (coding or non-coding) can be achieved using a library of guide RNAs (gRNAs) to direct Cas9-mediated double-stranded breaks; error-prone repair at these sites by non-homologous end-joining (NHEJ) generates loss-of-function alleles. Alternatively, a precise, intended mutation can be generated when designed replacement DNA cassettes are supplied and the break is repaired by homology-directed repair; however, previous applications have involved libraries of replacement cassettes for mutagenesis of single loci.

Garst *et al.* sought to combine these strategies to engineer precise designed mutations on a genomic scale. They generated a library of >50,000 plasmids containing 3 variable but covalently coupled components: a gRNA-expressing region, a replacement cassette to generate a defined mutation at the particular gRNA target site, and a barcode sequence to uniquely identify the overall construct. As an additional feature, the replacement cassette was designed to generate the main target-site mutation, as well as a synonymous mutation in a nearby protospacer-adjacent motif (PAM) to minimize further Cas9 cleavage at edited loci.

In initial proof-of-principle tests, the team transfected single constructs or pooled libraries of constructs into Cas9-expressing *Escherichia coli* cells and achieved ~70% efficiency of correct editing at target loci. They then tested single-gene CREATE libraries for functional screening: mutagenesis of *folA* (which encodes dihydrofolate reductase) to screen for trimethoprim resistance and mutagenesis of the *acrB* drug efflux pump to screen for isobutanol resistance. In both cases, sequencing across the barcode region in populations of *E. coli* cells was used to infer genome edits that were relatively enriched in the treated samples, and follow-up validation experiments using individual constructs confirmed that these edits indeed conferred treatment resistance.

Turning to multigenic and genome-scale applications, Garst *et al.* investigated thermotolerance in *E. coli*. Using a library based on known thermotolerance mutations from a previous long-term experimental evolution study, as well as the full genome-scale library of >50,000 constructs, they identified known and novel thermotolerant mutations. Key benefits over standard experimental evolution approaches are the rapid generation of mutations (in contrast to the months that they can take to arise naturally), the relatively straightforward linking of phenotypic effects to an identified mutation rather than potential background mutations, and the ease of generating and testing more-complex mutations beyond single-nucleotide variants.

A strength of the CREATE system is the minimal genomic changes that it induces, as only the target-site mutation and the PAM are targeted for mutation; this should minimize spurious phenotypes arising from disruptions elsewhere in the genome. However, as this setup involves readout based on sequencing an extrachromosomal plasmid barcode, it will be interesting to determine whether adapting CREATE to genomically integrate a barcode will provide benefits. In particular, an integrated barcode would enable a more quantitative and long-term assessment of the number of cells with each genotype in the population. The authors report preliminary success of plasmid-based CREATE in yeast, but genomically integrated systems (and strategies to minimize NHEJ) are likely to be required for applications in higher eukaryotic systems.

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ORIGINAL ARTICLE Garst, A. D. *et al.* Genome-wide mapping of mutations at single-nucleotide resolution for protein, metabolic and genome engineering. *Nat. Biotechnol.* http://dx.doi.org/10.1038/nbt.3718 (2016)

FURTHER READING Shalem, O., Sanjana, N. E. & Zhang, F. High-throughput functional genomics using CRISPR–Cas9. *Nat. Rev. Genet.* **16**, 299–311 (2015)

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