CRISPR-based modulation of bacterial genome stability

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One of the major results of the ”postgenomic era” is the possibility to genetically reprogram various organisms to fulfill societal needs. Eubacteria are often the targets of genetic engineering due to their easy and rapid culturing, the availability of full genome sequences, and a rich toolbox at hand for their genetic modification. Applications of engineered bacteria are numerous, including the production, breakdown, or sensing of certain chemical compounds. These functions of interest are usually unstable, however, due to at least two factors: i) the spontaneous mutations of the gene circuits encoding the added function, and ii) a continuous selective pressure to lose the function of interest due to the fitness burden that these extra functions pose on the engineered cells. There are multiple strategies under development to circumvent this problem, many belonging to the large group of mutation-rate reduction. Several bacterial strains have been engineered that display a lowered rate of point mutations or deletions. Reducing or eliminating insertional mutagenesis however, has been tedious and challenging, requiring the serial elimination of resident mobile genetic elements and the iterative verification of the altered genotype. To evade such difficulties, we demonstrate here a plasmid-based, portable tool for the easy and rapid downregulation of transposable element-mobility in various strains of *Escherichia coli*. Our system applies the non-cleaving, dCas9 enzyme of *Streptococcus pyogenes* to constitutively bind to the left inverted repeats of several insertion sequences (IS) in parallel. We demonstrate that in the case of most IS types this results in the transcriptional downregulation of the transposase of the given element. In all cases nevertheless, we observed the reduction or complete elimination of IS mobility. As a result of the IS-silencing process, a lowered mutation rate could be detected both at chromosomal and at plasmid-borne loci. Overall, we showcase a rapidly transferable system that can increase bacterial genetic stability and therefore extend the half-life of industrially relevant functions of engineered bacterial strains.