Transient overexpression of DNA adenine methylase enables efficient and mobile genome engineering with reduced off-target effects

Homologous recombination of single-stranded oligonucleotides is a highly efficient process for introducing precise mutations into the genome of E. coli and other organisms when mismatch repair (MMR) is disabled. This can result in the rapid accumulation of off-target mutations that can mask desired phenotypes, especially when selections need to be employed following the generation of combinatorial libraries. While the use of inducible mutator phenotypes or other MMR evasion tactics have proven useful, reported methods either require non-mobile genetic modifications or costly oligonucleotides that also result in reduced efficiencies of replacement. Therefore a new system was developed, Transient Mutator Multiplex Automated Genome Engineering (TM-MAGE), that solves problems encountered in other methods for oligonucleotide-mediated recombination. TM-MAGE enables nearly equivalent efficiencies of allelic replacement to the use of strains with fully disabled MMR and with an approximately 12- to 33-fold lower off-target mutation rate. Furthermore, growth temperatures are not restricted and a version of the plasmid can be readily removed by sucrose counterselection. TM-MAGE was used to combinatorially reconstruct mutations found in evolved salt-tolerant strains, enabling the identification of causative mutations and isolation of strains with up to 75% increases in growth rate and greatly reduced lag times in 0.6 M NaCl.