Multiplex metabolic pathway engineering using CRISPR/Cas9 in Saccharomyces cerevisiae

CRISPR/Cas9 is a simple and efficient tool for targeted and marker-free genome engineering. Here, we report the development and successful application of a multiplex CRISPR/Cas9 system for genome engineering of up to 5 different genomic loci in one transformation step in baker's yeast Saccharomyces cerevisiae. To assess the specificity of the tool we employed genome re-sequencing to screen for off-target sites in all single knock-out strains targeted by different gRNAs. This extensive analysis identified no more genome variants in CRISPR/Cas9 engineered strains compared to wild-type reference strains. We applied our genome engineering tool for an exploratory analysis of all possible single, double, triple, quadruple and quintuple gene disruption combinations to search for strains with high mevalonate production, a key intermediate for the industrially important isoprenoid biosynthesis pathway. Even though we did not overexpress any genes in the mevalonate pathway, this analysis identified strains with mevalonate titers greater than 41-fold compared to the wild-type strain. Our findings illustrate the applicability of this highly specific and efficient multiplex genome engineering approach to accelerate functional genomics and metabolic engineering efforts. (C) 2015 International Metabolic Engineering Society. Published by Elsevier Inc. All rights reserved.