CrEdit: CRISPR mediated multi-loci gene integration in Saccharomyces cerevisiae

Background: One of the bottlenecks in production of biochemicals and pharmaceuticals in Saccharomyces cerevisiae is stable and homogeneous expression of pathway genes. Integration of genes into the genome of the production organism is often a preferred option when compared to expression from episomal vectors. Existing approaches for achieving stable simultaneous genome integrations of multiple DNA fragments often result in relatively low integration efficiencies and furthermore rely on the use of selection markers. Results: Here, we have developed a novel method, CrEdit (CRISPR/Cas9 mediated genome Editing), which utilizes targeted double strand breaks caused by CRISPR/Cas9 to significantly increase the efficiency of homologous integration in order to edit and manipulate genomic DNA. Using CrEdit, the efficiency and locus specificity of targeted genome integrations reach close to 100% for single gene integration using short homology arms down to 60 base pairs both with and without selection. This enables direct and cost efficient inclusion of homology arms in PCR primers. As a proof of concept, a non-native beta-carotene pathway was reconstructed in S. cerevisiae by simultaneous integration of three pathway genes into individual intergenic genomic sites. Using longer homology arms, we demonstrate highly efficient and locus-specific genome integration even without selection with up to 84% correct clones for simultaneous integration of three gene expression cassettes. Conclusions: The CrEdit approach enables fast and cost effective genome integration for engineering of S. cerevisiae. Since the choice of the targeting sites is flexible, CrEdit is a powerful tool for diverse genome engineering applications.
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