Genome-scale Engineering of *Saccharomyces cerevisiae* with Single Nucleotide Precision

Zehua Bao,[1] Pu Xue,[2]* (puxue2@illinois.edu), Mohammad HamediRad,[2] Han Xiao,[1,3] Ipek Tasan,[4] Ran Chao,[4] Jing Liang,[5] and **Huimin Zhao**[1,2,4,5,6,★]

[1]Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801; [2]Department of Chemical and Biomolecular Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801; [3]Current address: State Key Laboratory of Microbial Metabolism, Joint International Research Laboratory of Metabolic & Developmental Sciences, and Laboratory of Molecular Biochemical Engineering, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, China; [4]Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL 61801; [5]Metabolic Engineering Research Laboratory, Science and Engineering Institutes, Agency for Science, Technology and Research, Singapore 138669, Singapore; and [6]Departments of Chemistry and Bioengineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801

[★]Correspondence should be addressed to H.Z. (zhao5@illinois.edu). Phone: (217) 333-2631. Fax: (217) 333-5052.

http://scs.illinois.edu/~zhaogrp/

Project Goals:

To develop an efficient, iterative, genome-scale single nucleotide resolution editing tool to improve complex yeast phenotypes.

Abstract:

Through systematically exploring the fitness landscape in a genome-wide manner, genome-scale engineering can test multiple hypotheses in parallel and overcome our limited knowledge of biocomplexity. However, existing methods to create a genome-wide deletion library in yeast require years and many researchers. Here we report a CRISPR/Cas9 and homology-directed repair assisted genome-scale engineering (CHAnGE) method that enables generation of a genome-wide library of yeast gene disruption mutants each with a defined 8 nucleotides removal by a single researcher within a month. Such unprecedented precise and small deletions minimize the risk of disrupting overlapping genomic features and chromatin structures. We validated CHAnGE by identification of single disruption mutants conferring 5~7 fold improved tolerance to different growth inhibitors including acetic acid and furfural in a genome-wide manner (targeting 6459 genes). We further iterated CHAnGE for directed evolution of yeast genomes, achieving an additional 3~8 fold improvement. Finally, we demonstrated the genome-editing capability of CHAnGE at single-nucleotide resolution and provided preliminary evidence that CHAnGE will work in mammalian cells.[1]

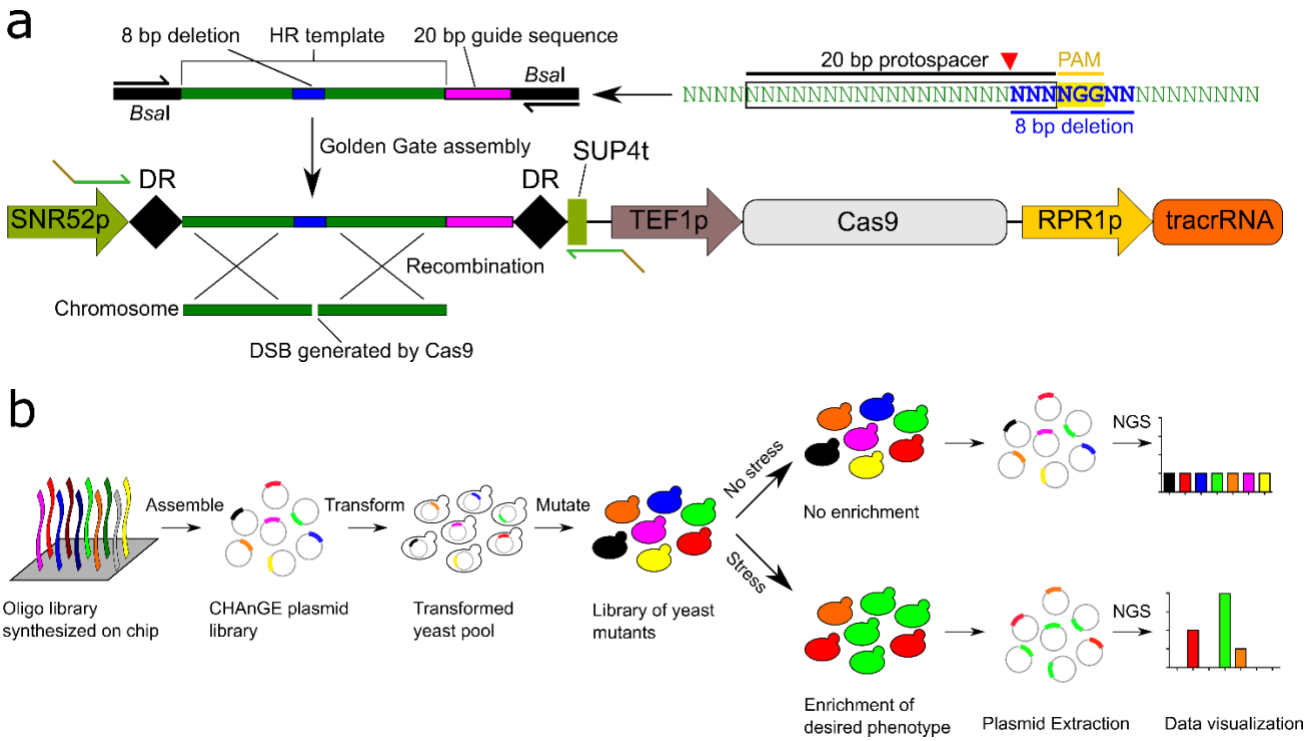

Figure 1. The CHAnGE workflow and validation. (a) Design of the CHAnGE oligonucleotide cassette. A 8 bp deletion near the Cas9 cleavage site (denoted by the red triangle) removes the PAM sequence and part of the protospacer sequence and introduces a frame shift mutation. A 100 bp HR template with 50 bp homology arms and the 8 bp deletion at the center was placed 5' of the guide sequence, constituting the CHAnGE cassette. The CHAnGE cassette was cloned into a Cas9 and tracrRNA expressing plasmid using Golden Gate assembly. After cleavage by Cas9, the target ORF was mutated by recombination with the HR template. **(b)** The CHAnGE workflow.[1]

References

1. Z. Bao, et al. "Genome-scale Engineering of *Saccharomyces cerevisiae* with Single Nucleotide Precision." *Nature Biotechnology* in press.

Funding statement

This material is based upon work supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research under Award Number DE-SC0018260.