BRC Science Highlight
March 2018

Multiplex Genome Engineering of Polyploid Industrial Yeast Strains using an Optimized CRISPR/Cas9 System

Background/objective

- Compared with lab strains, industrial yeast strains have the advantages of higher productivity and robustness under harsh industrial conditions.
- Although CRISPR/Cas9 has been widely used for industrial yeast strain engineering, its application in industrial yeast strains is less successful (15-60% efficiency for single gene deletion in ATCC 4124).

Approach

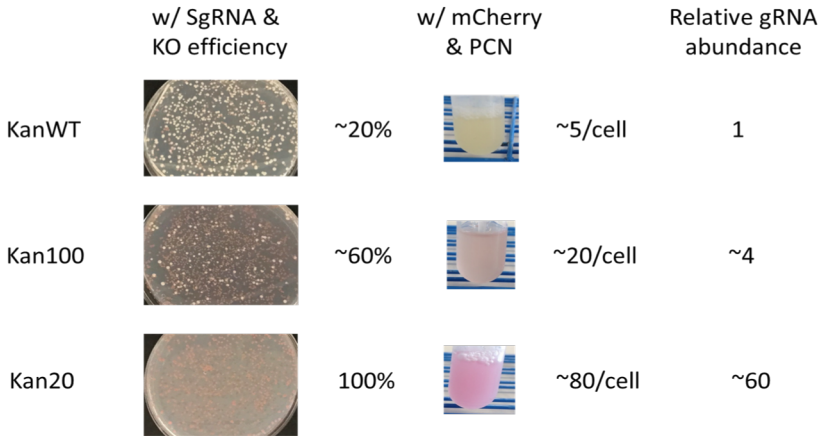
- Increasing gRNA abundance via super-high copy number plasmids.

Results

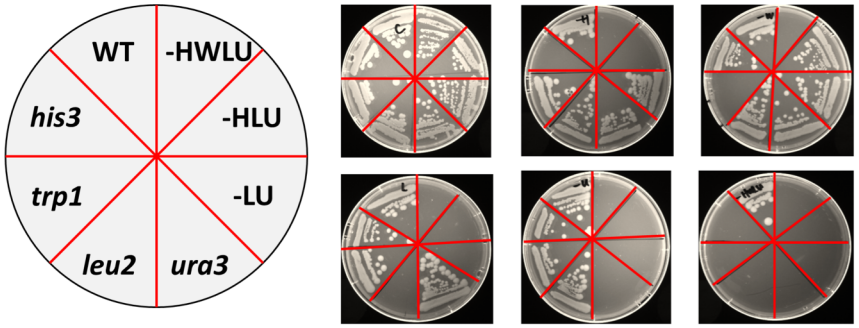
- Plasmids with higher copy numbers increased gRNA levels, resulting in higher genome editing efficiencies (up to 100% efficiency).
- Up to 12 alleles were disrupted in a single step with 100% knock-out efficiency.

Significance

- Facile engineering of industrial yeast strains for practical applications, i.e. biofuel production.



Plasmids with increased copy numbers for multiplex genome engineering of industrial yeast strains.



All the single, double, triple, and quadruple knock-out strains were constructed in a single step with 100% efficiency.

[1] *Lian, et al. 2017. "Engineered CRISPR/Cas9 system for multiplex genome engineering of polyploid industrial yeast strains."* ***Biotechnology and Bioengineering****, DOI: 10.1002/bit.26569*

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