

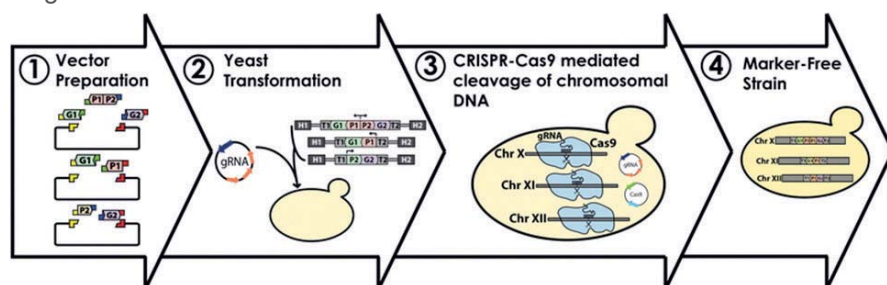
EXPLOITABLE FOREGROUND

Genetic engineering toolbox for manipulation of industrial yeast strains

Explanation and Purpose

Polyploid industrial strains of *Saccharomyces cerevisiae* can be rapidly engineered using the provided genetic toolbox. The toolbox comprises a set of integrative vectors that target eleven specific well-characterized genomic locations. The vectors allow for selection in prototrophic yeast strains using six different dominant selection markers. The markers can subsequently be removed using *loxP/creA* recombination. Alternatively, the integration is ensured by CRISPR/Cas9 system. The vectors allow for efficient overexpression of multiple genes.

CRISPR/Cas vectors can also be employed for gene deletions and other genome edits, including combinations of several different genome edits in a single transformation event.



Exploitation Strategy

The vector toolbox has been distributed to 30+ academic and industrial laboratories. The vectors are available for research use via public depository – Addgene, including user guidelines:

<https://www.addgene.org/kits/borodina-easyclone-v2/>
<https://www.addgene.org/kits/borodina-easyclone-markerfree/>
<https://www.addgene.org/browse/article/22359/>

For commercial use, the standard terms of Technical University of Denmark apply.

Detailed description of the toolbox is included in the following research papers:

1. Stovicek V., Borodina I., Forster J. (2015): CRISPR-Cas system enables fast and simple genome editing of industrial *Saccharomyces cerevisiae* strains. *Metabolic Engineering Communications* 2:13-22
2. Stovicek V., Borja G., Forster J., Borodina I. (2015): EasyClone 2.0: Expanded toolkit of integrative vectors for stable gene expression in industrial *Saccharomyces cerevisiae* strains. *Journal of Industrial Microbiology and Biotechnology* 42(11):1519-1531
3. Jessop-Fabre MM, Jakočiūnas T, Stovicek V, Dai Z, Jensen MK, Keasling J, Borodina I. (2016): EasyClone-MarkerFree: A vector toolkit for markerless integration of genes into *Saccharomyces cerevisiae*. *Biotechnol J* 1(8):1110-1117

IPR Measures

For commercial exploitation, refer to general CRISPR patents (if any) in the given country.

Impact of Exploitation

Industrial strains of *S. cerevisiae* can be engineered rapidly and efficiently using the developed genetic toolbox.

Development of 2nd Generation Biorefineries - Production of Dicarboxylic Acids and Bio-based Polymers Derived Thereof

bioREFINE-2G

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