CRISPR INTERFERENCE TECHNOLOGY FOR DEVELOPMENT OF MORE TOLERANT INDUSTRIAL YEAST STRAINS

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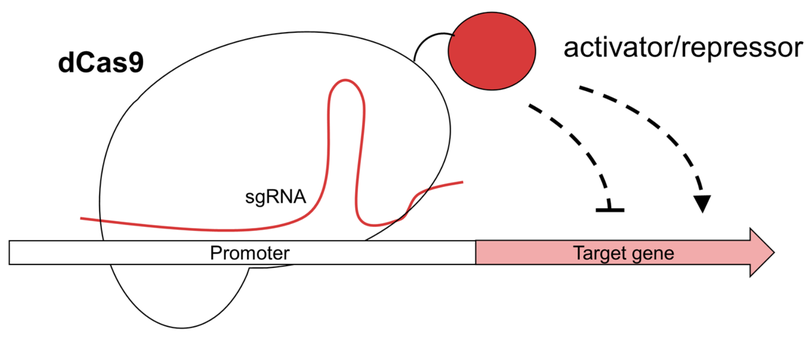
CRISPRi/a TECHNOLOGY

The CRISPR interference / activation (CRISPRi/a) technology utilizes a catalytically inactive Cas9 (dCas9) to modulate the expression of genes targeted by an sgRNA, allowing the alteration of gene expression without altering the gene target sequence.



INDUSTRIAL STRAIN KE6-12

Yeast strain optimized for ethanol production from lignocellulosic hydrolysates

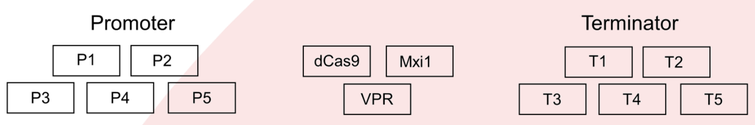
Polyploid; optimized for xylose consumption by evolutionary engineering

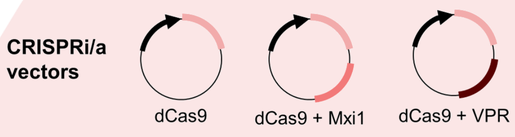
Is the CRISPRi/a technology suitable for industrial strains?

1 Design of CRISPRi/a vectors

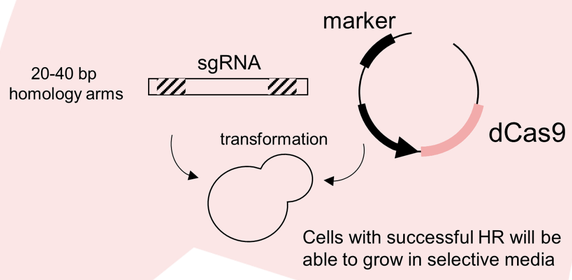
a) Vector construction

Assembly of vectors expressing dCas9 + activator or repressor by modular cloning (Moclo Yeast Toolkit).



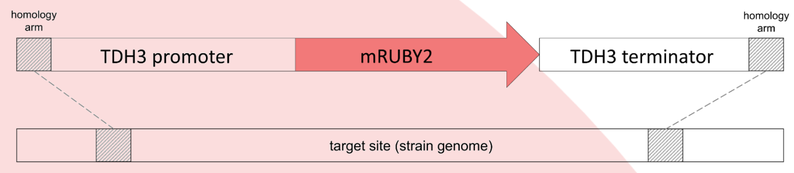


b) Transformation strategy

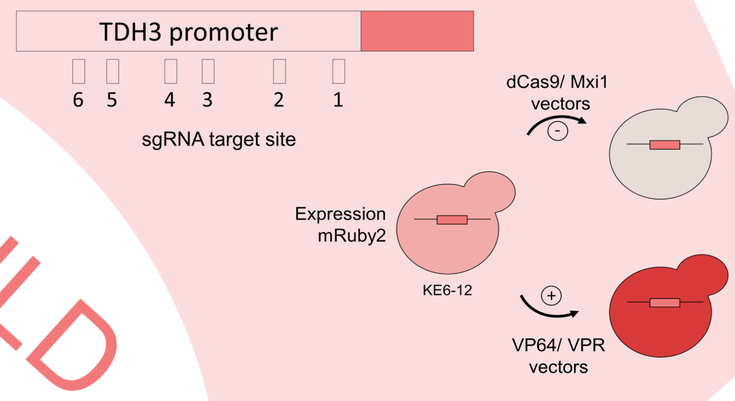


2 Proof of Concept

a) Insertion of a red fluorescent gene by CRISPR/Cas9.

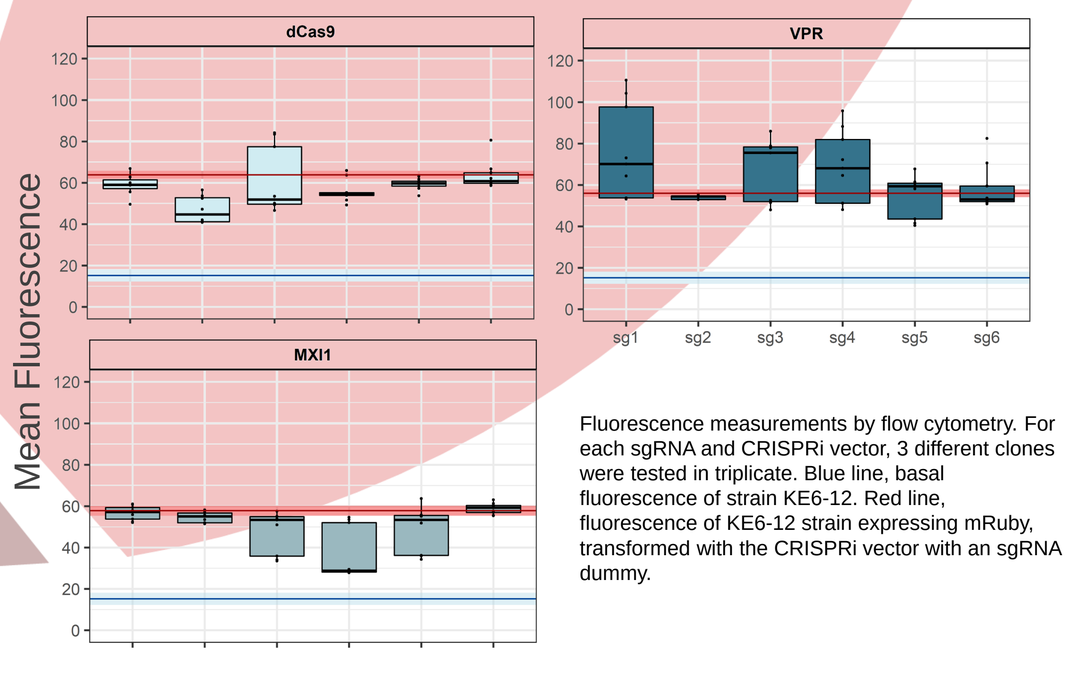


b) Transformation CRISPRi/a vectors + promoter targeting in different positions.



3 Strain analysis

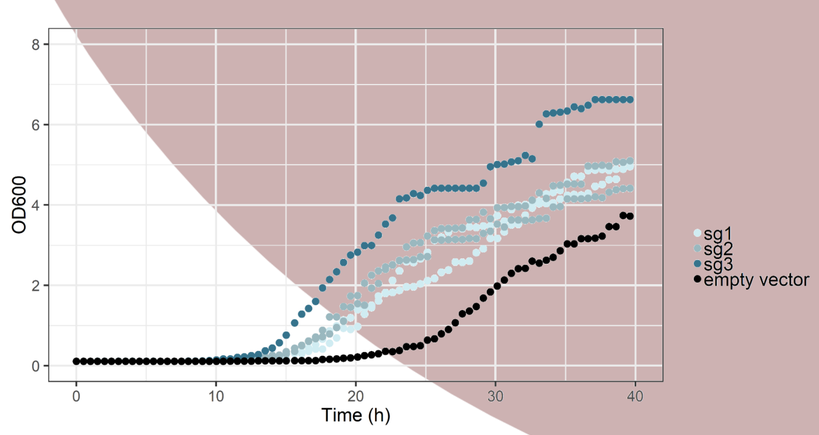
sgRNAs 3, 4 and 5 (-277, -351 and -469 bp relative to the promoter start) had a major impact on the strain expressing various CRISPRi vectors.
A higher repression of mRuby2 was achieved with vectors containing dCas9-Mxi1 whereas in **strains with dCas9-VPR** the red expression of mRuby was **highly upregulated**.



Fluorescence measurements by flow cytometry. For each sgRNA and CRISPRi vector, 3 different clones were tested in triplicate. Blue line, basal fluorescence of strain KE6-12. Red line, fluorescence of KE6-12 strain expressing mRuby, transformed with the CRISPRi vector with an sgRNA dummy.

4 CRISPRi improving tolerance

Downregulation of **SSK2** (gene involved in the Hog1 apoptotic pathway) has been shown to confer furfural resistance in laboratory yeast strains. When targeted by dCas-Mxi1, the **industrial strain showed an improvement in growth in minimal media supplemented with 20mMof furfural**, compared to the control.



The growth of the strains was tested in the Growth Profiler platform (Enzyscreen), where the cell density was measured through imaging over time.

DESIGN BUILD TEST LEARN

- **The CRISPRi technology was successfully implemented in an industrial strain.**
- **The change in expression achieved was highly dependent on the sgRNA and dCas9-variant used.**
- **Downregulation of SSK2 improves furfural tolerance in the industrial strain KE6-12.**

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