

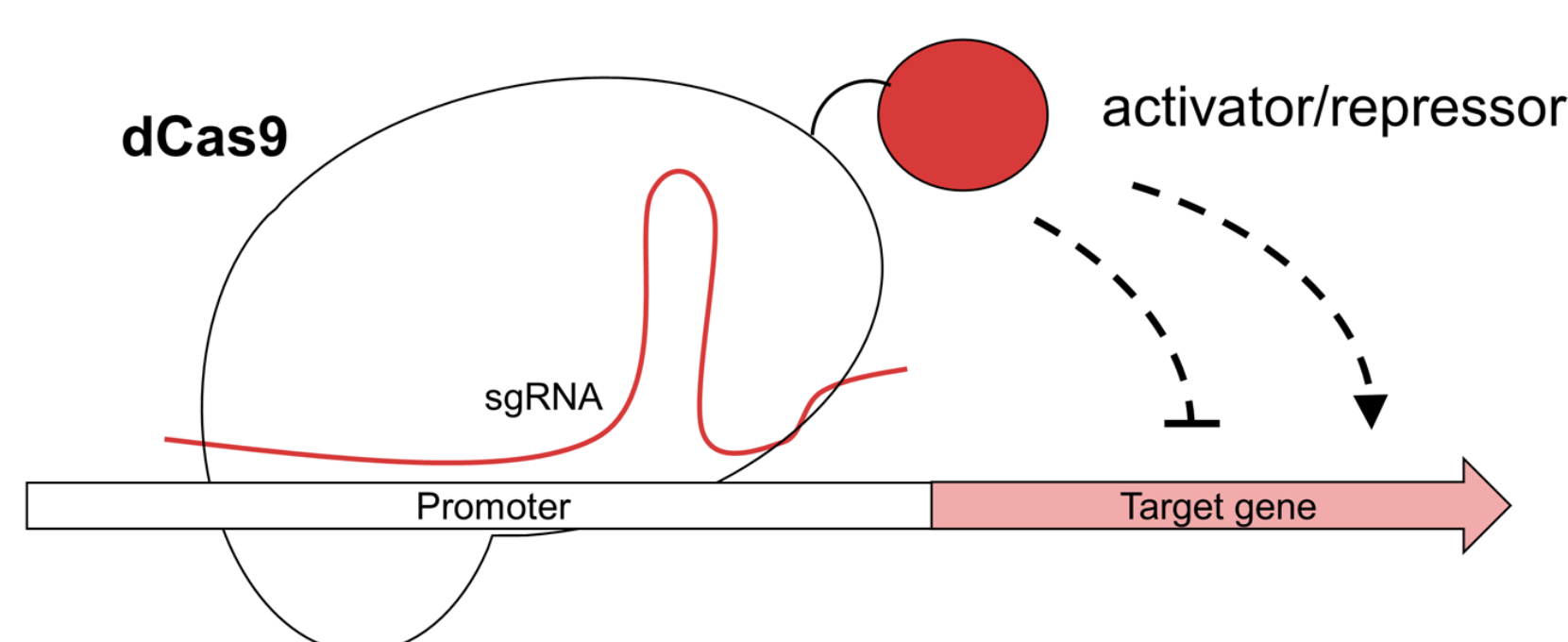


CRISPR INTERFERENCE TECHNOLOGY FOR DEVELOPMENT OF MORE TOLERANT INDUSTRIAL YEAST STRAINS

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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

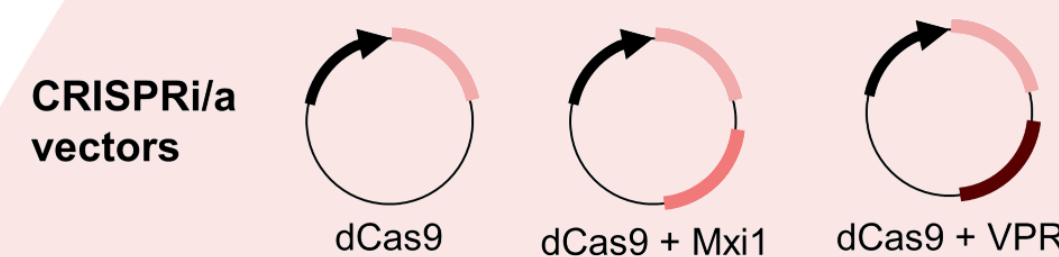
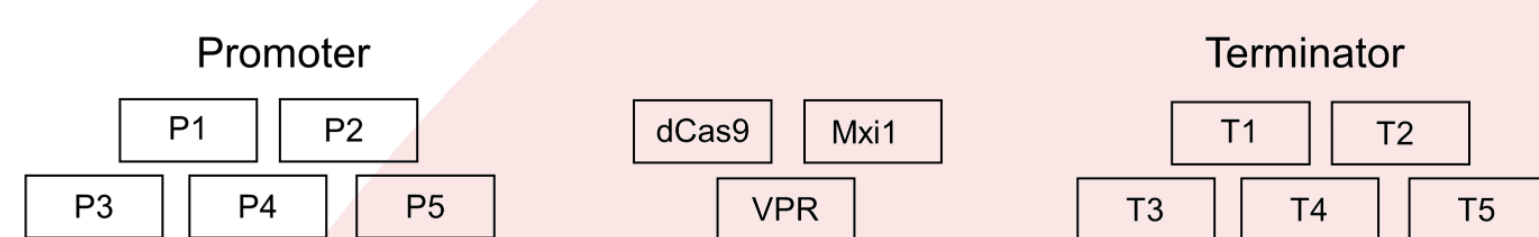


Is the CRISPRi/a technology suitable for industrial strains?

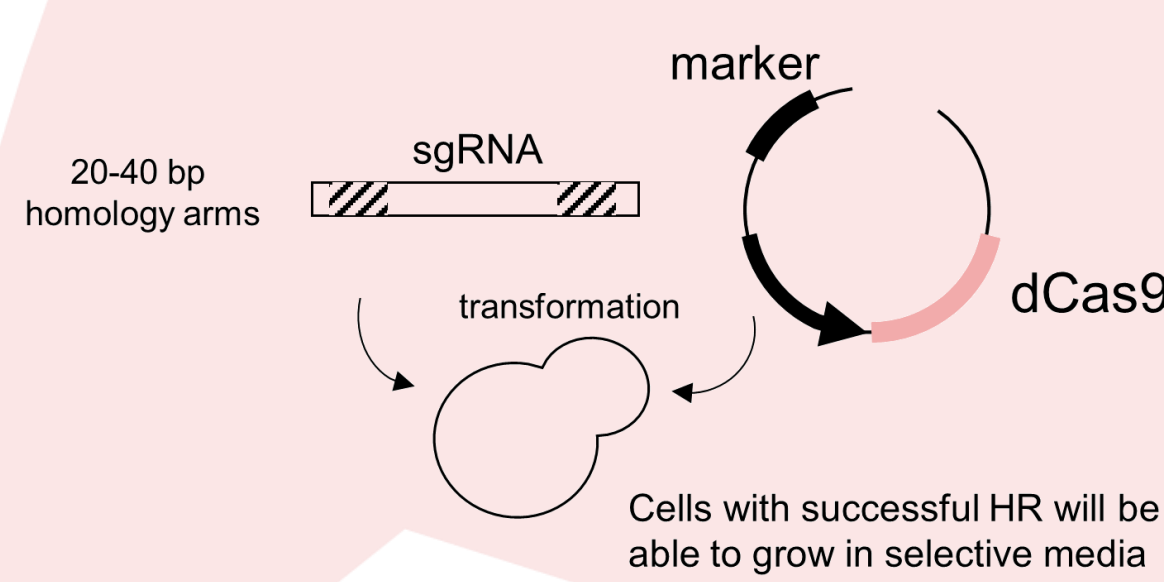
1 Design of CRISPRi/a vectors

a) Vector construction

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

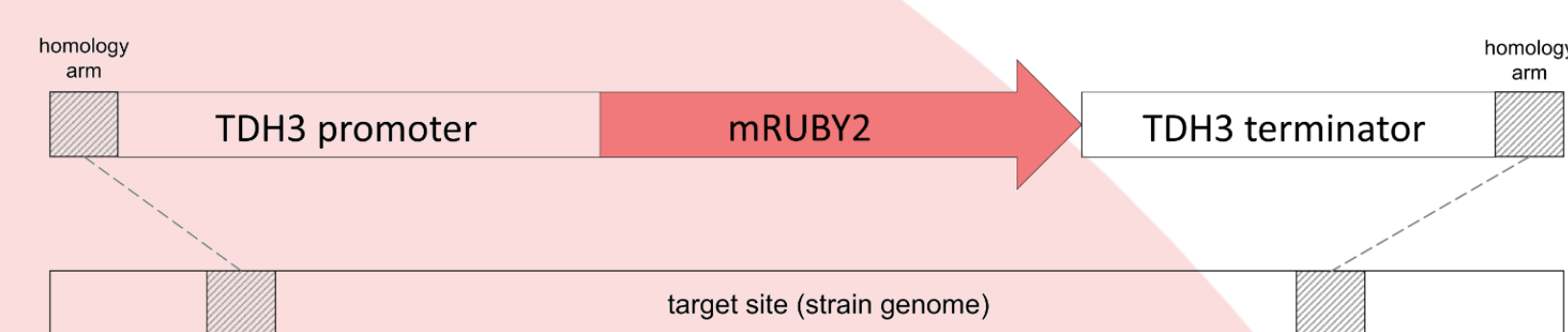


b) Transformation strategy

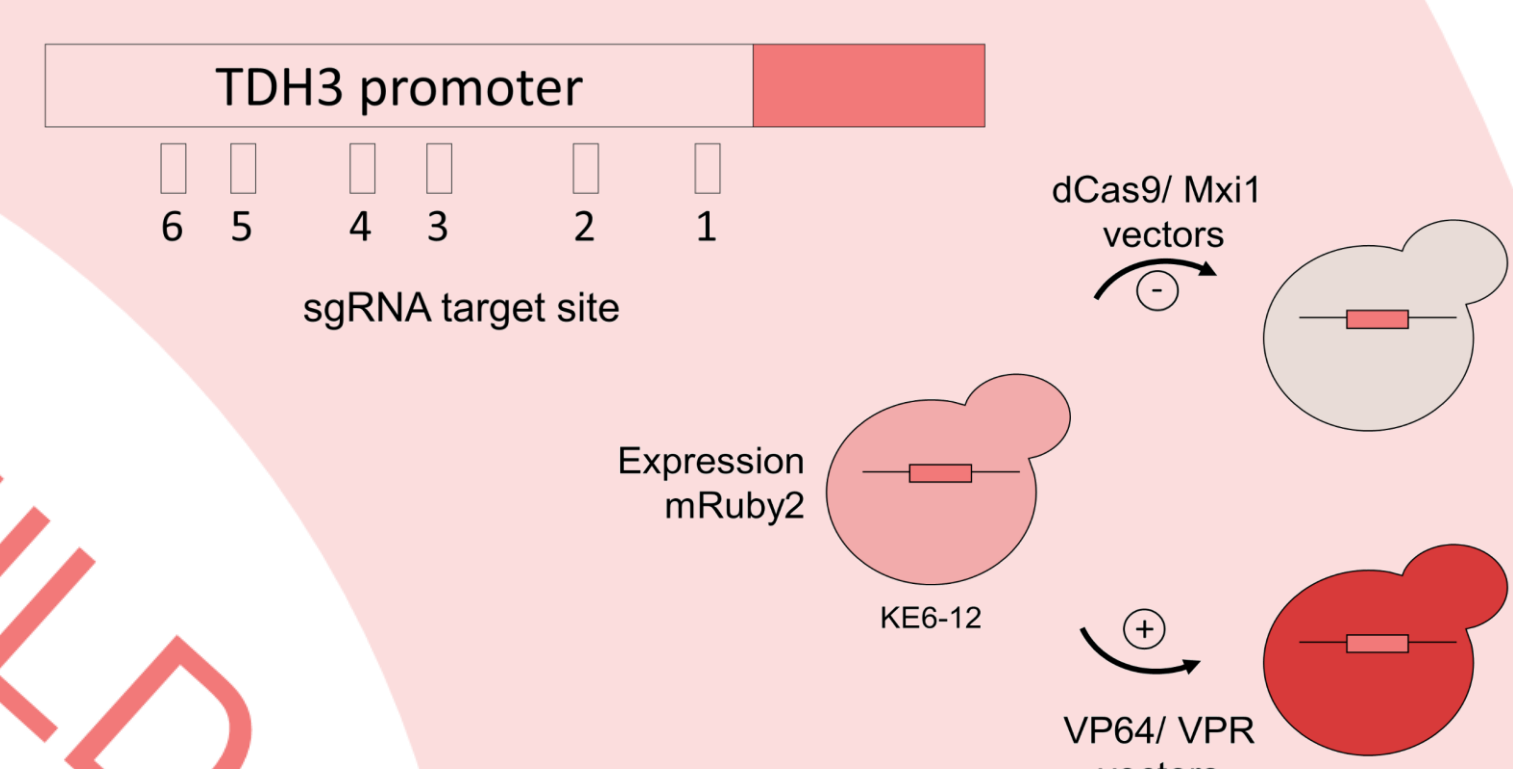


2 Proof of Concept

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

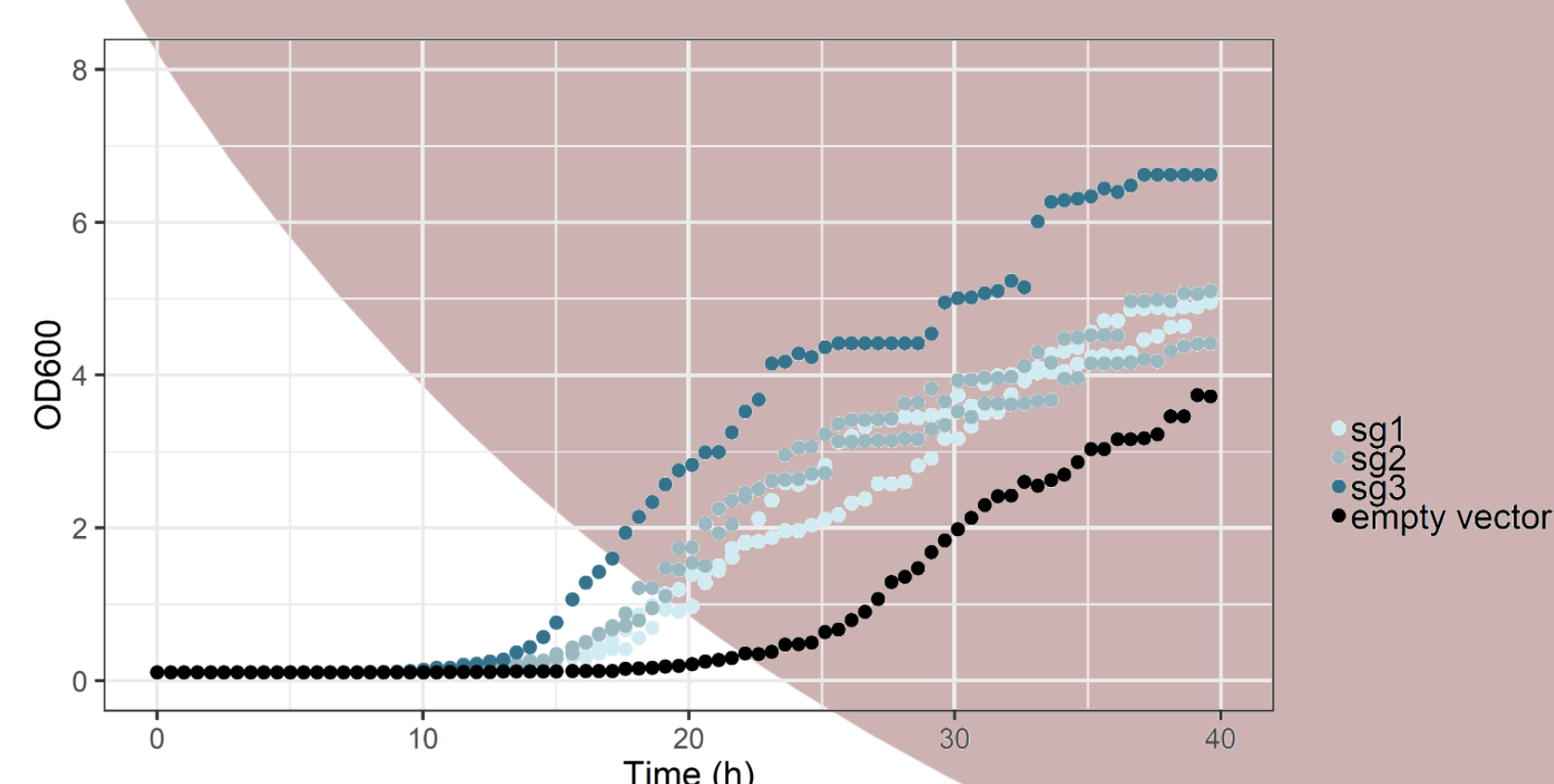


b) Transformation CRISPRi/a vectors + promoter targeting in different positions.



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 20mM of furfural**, compared to the control.

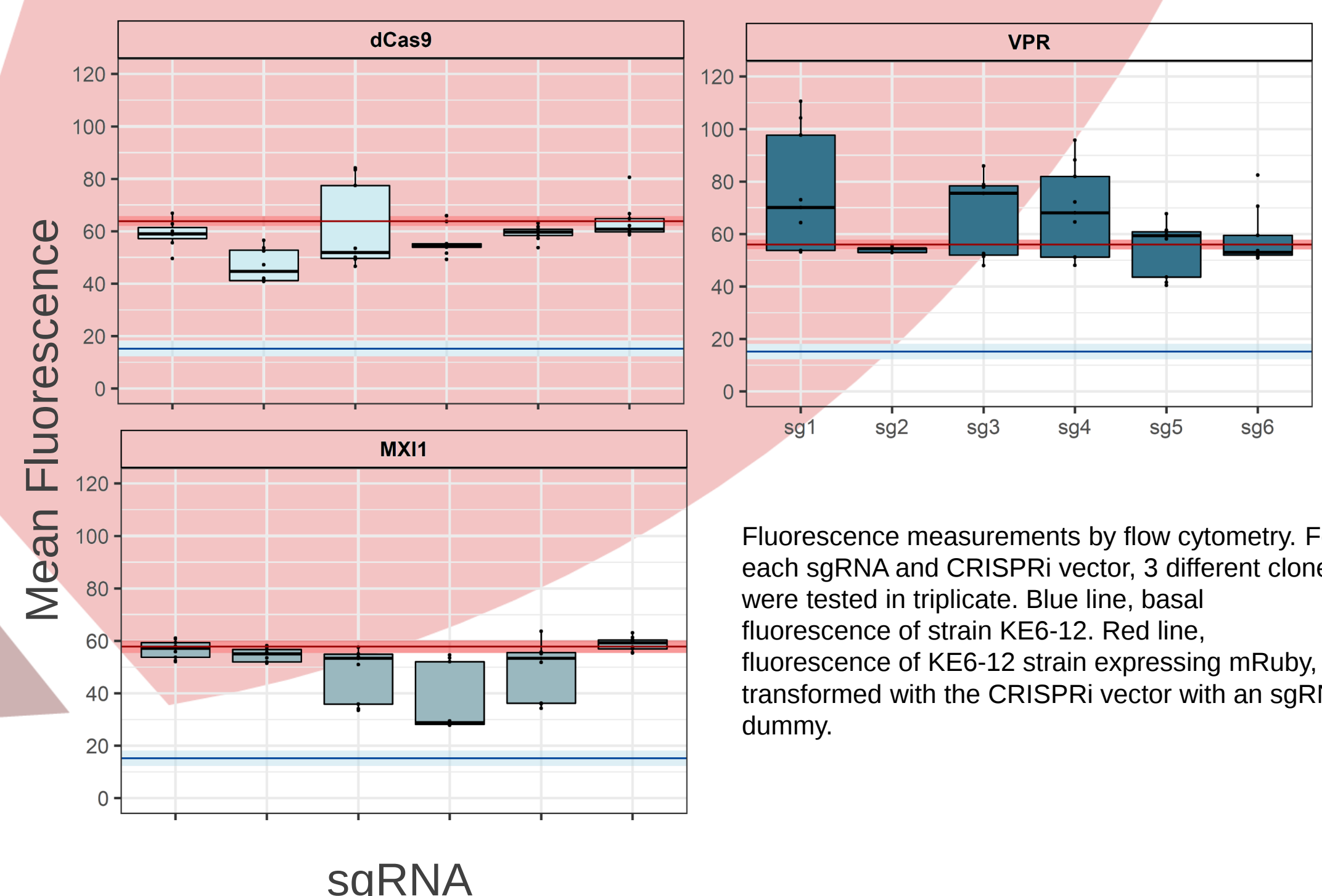


The growth of the strains was tested in the Growth Profiler platform (Enzyscreen), where the cell density was measured through imaging over time.

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.

- 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.