The CRISPR interference / activation (CRISPR/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 CRISPR/a technology suitable for industrial strains?

**1 Design of CRISPR/a vectors**

- **a) Vector construction**
  Assembly of vectors expressing dCas9 + activator or repressor by modular cloning (Moclo Yeast Toolkit)

- **b) Transformation strategy**
  Transformation CRISPR/a vectors + promoter targeting in different positions.

**2 Proof of Concept**

- **a) Insertion of a red fluorescent gene by CRISPR/Cas9.**
- **b) Transformation CRISPR/a vectors + promoter targeting in different positions.**

**3 Strain analysis**

- sgRNA 3, 4 and 5 (277, 351 and 469 bp relative to the promoter start) had a major impact on the strain expressing various CRISPR/a 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.

**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-Mxi, the industrial strain showed an improvement in growth in minimal media supplemented with 20Mof 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

- 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.