**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
   Transformation of KE6-12 strain with CRISPRi/a vectors

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**
   - sgRNA's 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.

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 dCas9-Mxi1, the industrial strain showed an improvement in growth in minimal media supplemented with 20Mm of furfural compared to the control.

**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.**