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?

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

Proof of Concept

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

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

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.

CRISPRi/a TECHNOLOGY

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.