

#### INDUSTRIAL BIOTECHNOLOGY

# Fine-Tuning the Stress Response of Saccharomyces cerevisiae using CRISPR interference Technology

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## **CRISPRi Technology**





> The CRISPRi technology can alter gene expression without

- permanent genetic alterations.
- > A catalytically inactive Cas9 protein (dCas9) fused to a repressor or an activator (such as VPR, a combination of 3 activation domains) can be targeted to a genetic locus using an sgRNA.
- $\succ$  dCas9 bound to the DNA can cause steric encumbrance, leading to gene repression, while dCas9 alone or fused to activation domains can lead to enhanced transcription activation.

## Workflow



Generation of CRISPRi mutants with a high-throughput method developed in our lab.

High-throughput screenings with 96-well plates in Growth Profiler 960 (Enzyscreen).

Generation of growth curves and growth parameters were extracted from the raw data.

Identification of yeast strains that are more robust towards lignocellulosic inhibitors.

#### Screening, 125 mM Acetic Acid



- $\geq$  Relative expression of HAA1 (blue), YPC1 (orange) and PDR1 (grey), after 5 hours.
- > Upregulation of HAA1, by dCas9-VPR+sgRNA1, targeting a region ~500 nt before the TSS of HAA1, decreased the lag phase in presence of acetic acid.
- > Down-regulation of PDR1 by Cas9+sgRNA3 and PDR1 disruption gave a slight increased robustness towards acetic acid.
- > Downregulation of HAA1, by dCas9-VPR+sgRNA2, targeting a region ~100 nt > Targeting PDR1 with dCas9+sgRNA4 at ~150 nt before the TSS

> The expression of YPC1 is regulated by HAA1. Expression is normalized to ACT1.

before the TSS of HAA1, drastically decreased tolerance to acetic acid.

 $\succ$  Expression of dCas9-VPR alone leads to a growth defect.

gave a slight decrease in tolerance towards acetic acid.

 $\succ$  Expression of dCas9 alone leads to a growth defect.

#### Conclusions

1. Modification of the native regulation of HAA1 and PDR1 with the CRISPRi technology can confer fitness benefits under acetic acid stress.

2. CRISPRi is a highly potential technique to study the stress regulation in yeast, as this technology is less disruptive compared to traditional disruption and overexpression methods.

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