Genetic engineering toolbox for industrial yeast

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Strain development for diacid production
Industrial yeast development

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Aim of the work

❍ Metabolic engineering of strains for production of wide variety of chemicals needs several rounds of genetic interventions

Industrial x laboratory yeast strains

- stress resistance
- robustness
- high fermentation capacity

- multiple ploidy, aneuploidy
- prototrophic strains
- low transformation efficiency

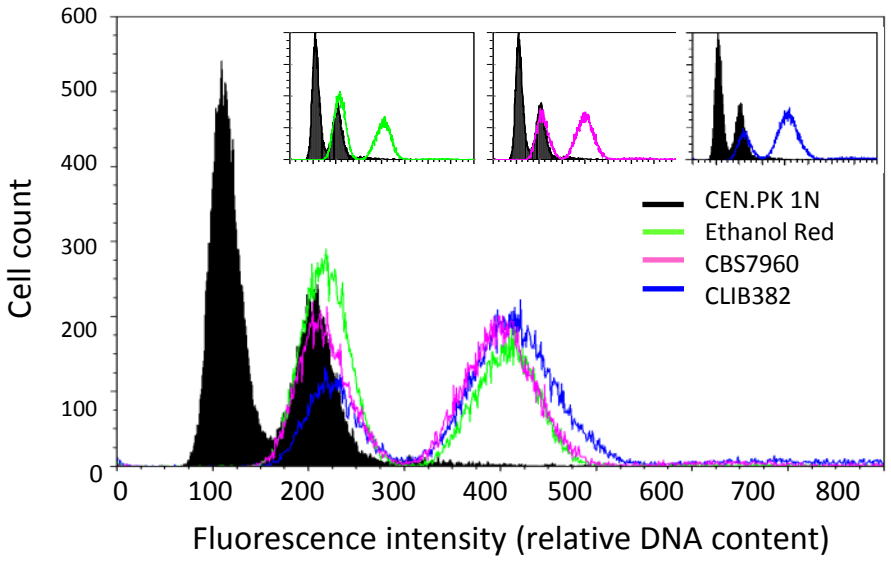
⇨ tools for efficient genome editing

❶ gene delivery & insertion

❷ gene disruption

Industrial *S. cerevisiae* strains

CBS7960	ethanol producer from cane-sugar syrup
Ethanol Red	industrial ethanol producer
CLIB382	Irish brewer's strain
CEN.PK	laboratory strain



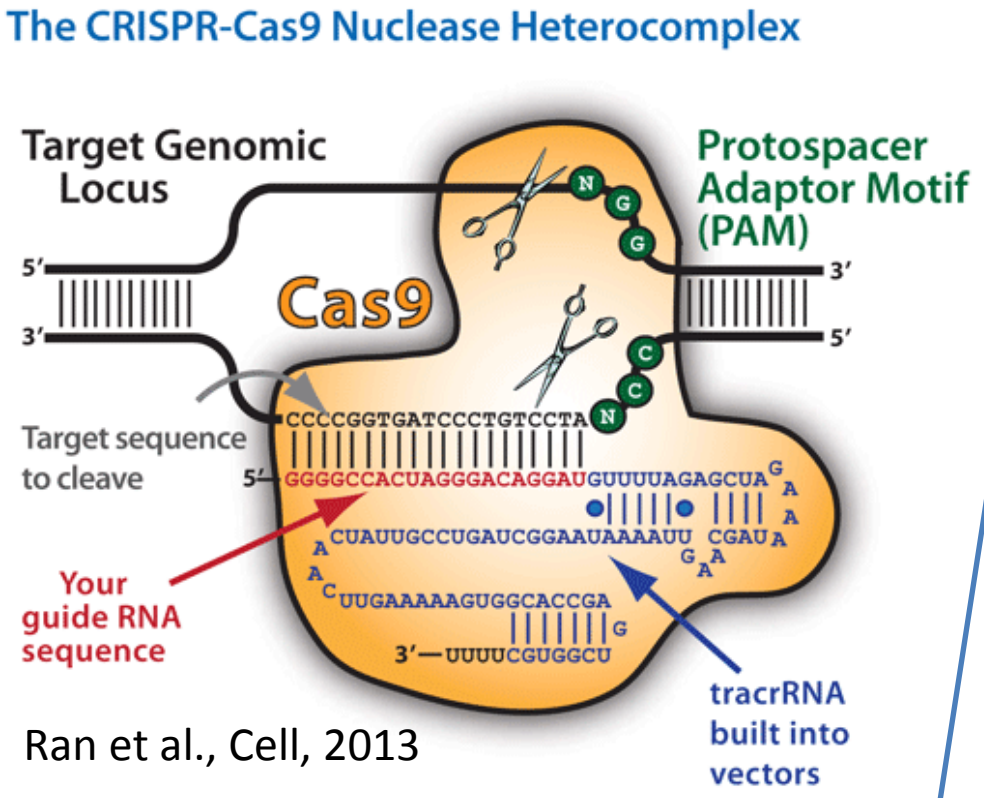
Activities

1) Development of genetic engineering toolbox for industrial *S. cerevisiae* strains

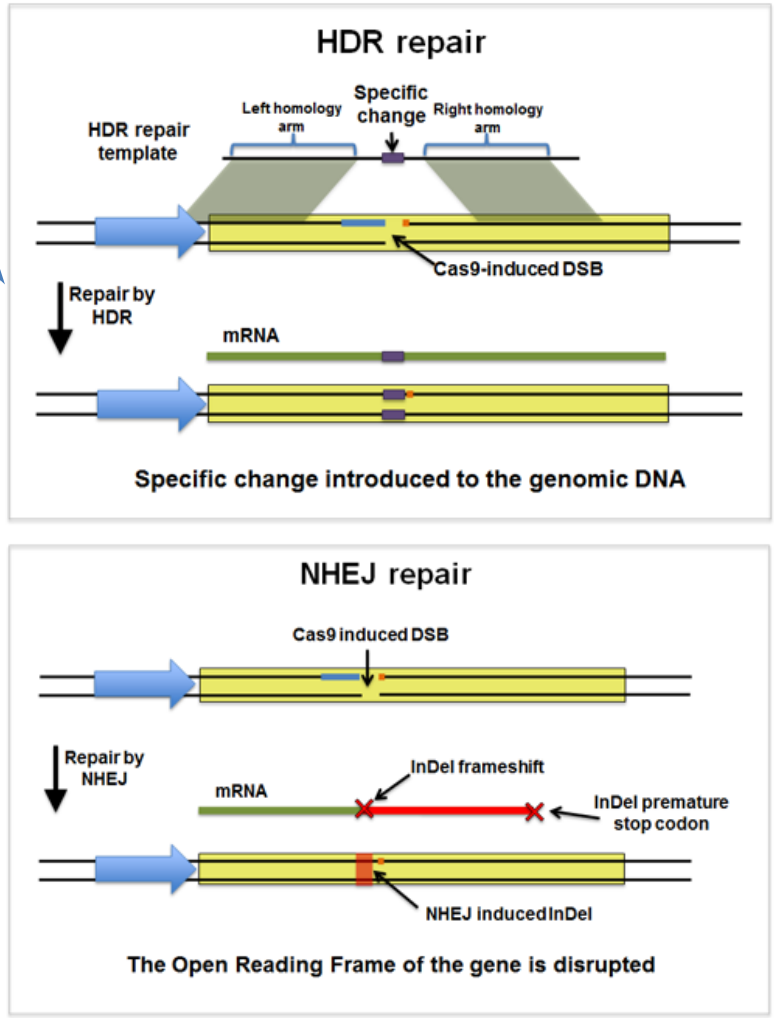
- fast and efficient strategy for gene disruption in industrial strains
- heterologous gene insertion strategy

CRISPR-Cas9 genome editing

- RNA guided specific endonuclease-mediated genome targeting

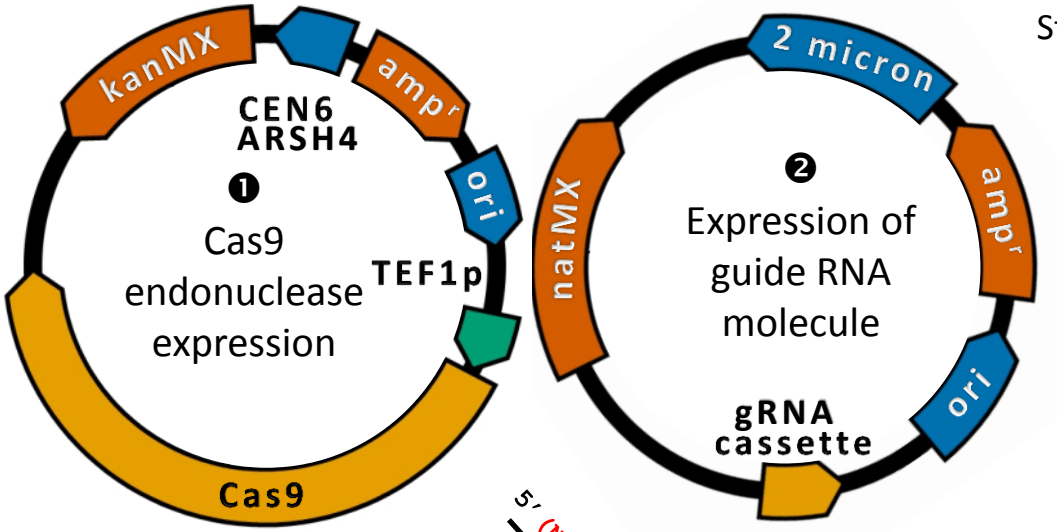


Ran et al., Cell, 2013

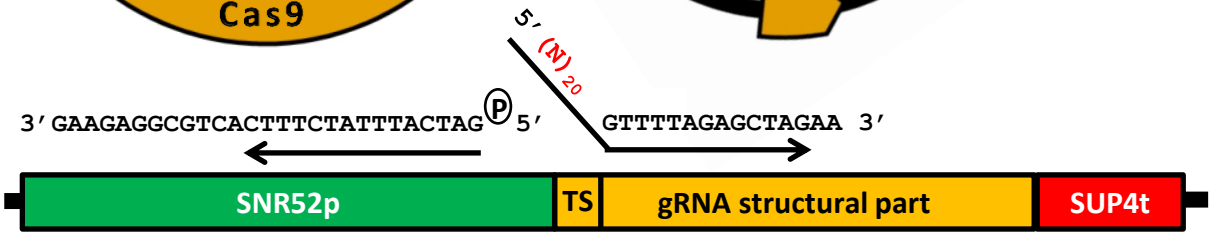


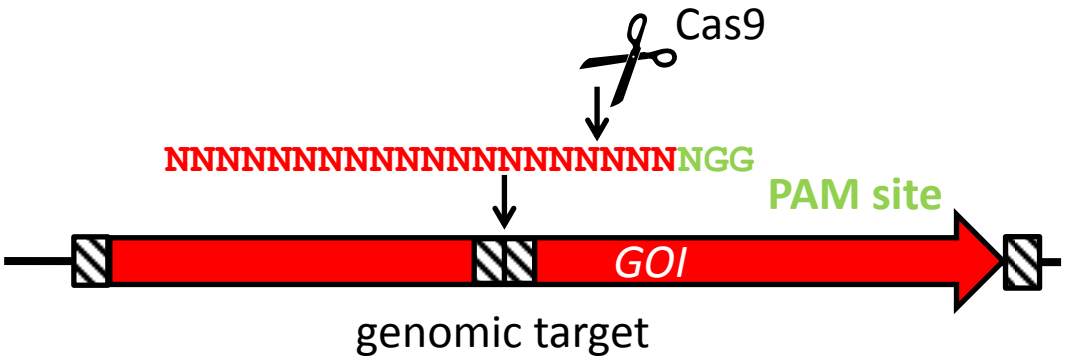
- Cas9 introduces DSB at specific genomic location
- Cas9 can be recruited to any sequence of choice

CRISPR-Cas9 system for industrial yeast gene targeting



Stovicek et al., 2015





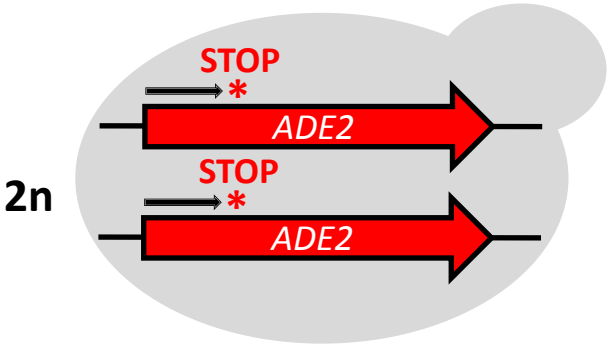
❸
Template for
DSB repair

A 90bp dsDNA oligo

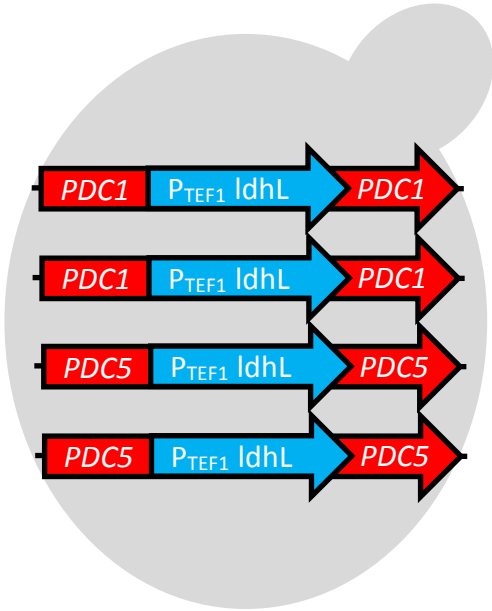
B insertion cassette

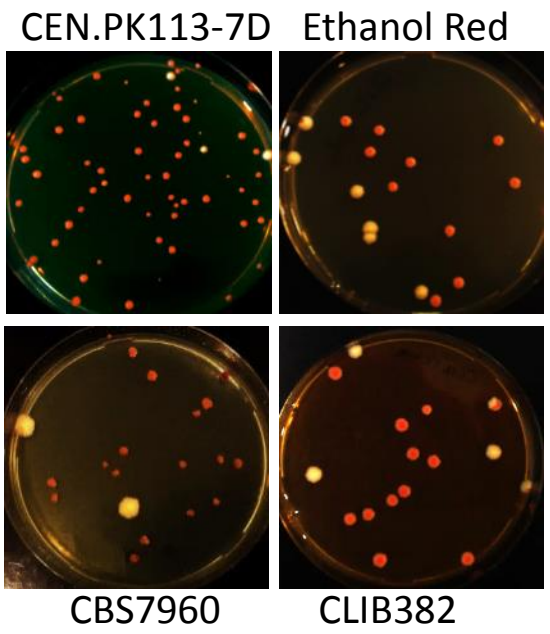
Proof-of-concept & case study

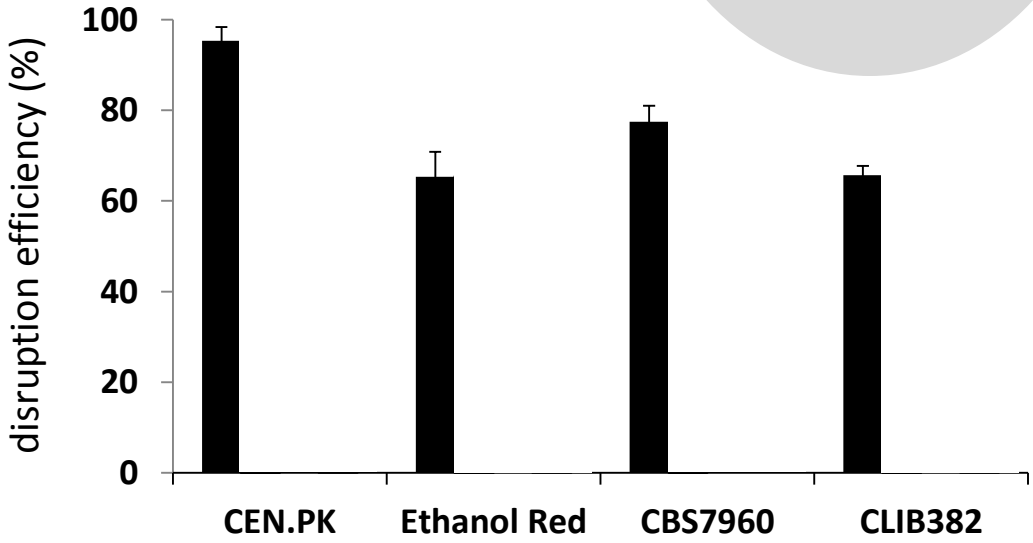
ADE2 disruption

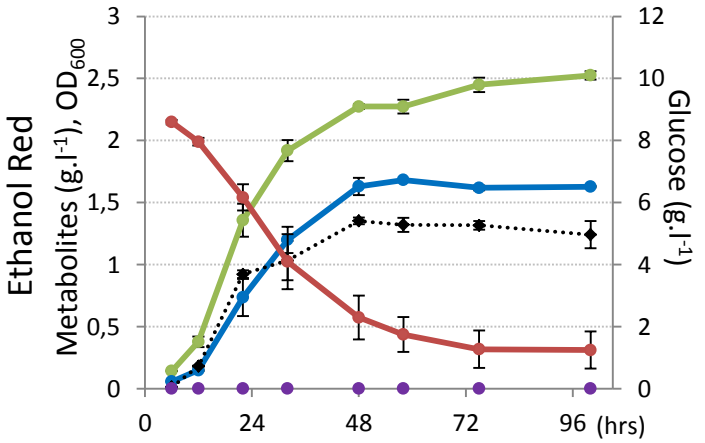


Lactate producing strain









Highlights & Conclusions

- **Highly efficient marker-free system for gene disruption in industrial yeast based on CRISPR-Cas9**
  - **demonstrated in metabolic engineering test case** ✓

Stovicek V., Borodina I., Förster J. (2015): CRISPR-Cas system enables fast and simple genome editing of industrial *Saccharomyces cerevisiae* strains. *Metabolic Engineering Communications 2:13-22*

- **New generation of integrative vectors suitable for gene integrations in industrial strains**
  - **demonstrated in metabolic engineering test case** ✓

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Thank you for your attention !!!

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