

Development of a CRISPR/Cas9-Based Tool for Gene Deletion in *Issatchenkia orientalis*

Background/objective

The non-model yeast, *Issatchenkia orientalis*, is a promising engineering candidate for the production of organic acids due to its high tolerance of low pH. However, lack of robust engineering tools currently hinders its further development for this purpose. Here, researchers developed an efficient CRISPR/Cas9-based tool for engineering of *I. orientalis*.

Approach

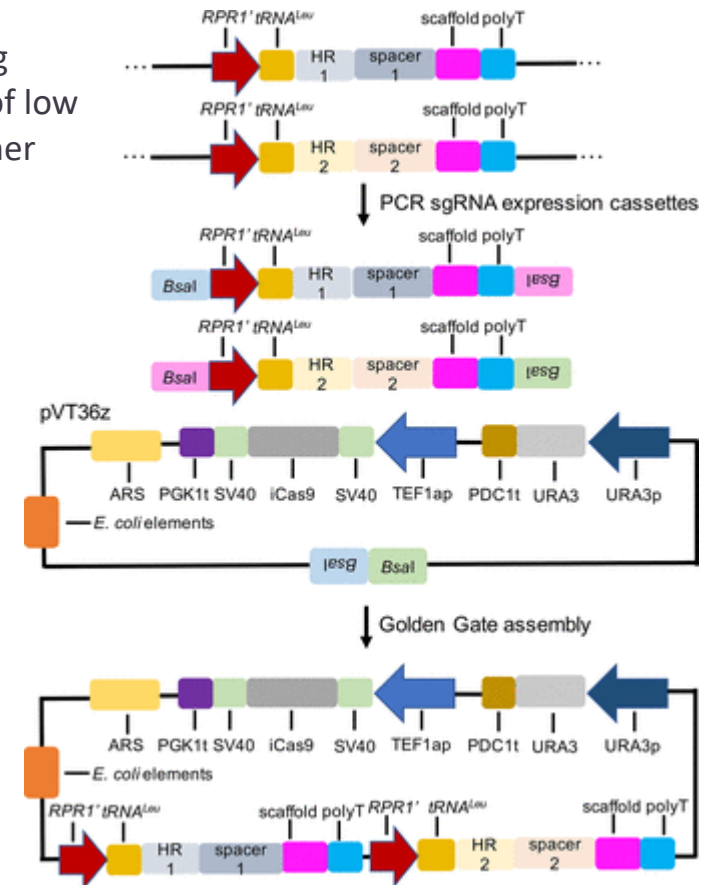
- ❖ GFP was used as a reporter to characterize ScARS function in *I. orientalis*.
- ❖ Single guide RNA expression was optimized by using different promoters.
- ❖ Single, double, and triple gene-disruption efficiencies were calculated.

Results

- ❖ The autonomously replicating sequence from *Saccharomyces cerevisiae* (ScARS) was functional for plasmid replication in *I. orientalis*, enabling efficient genome editing via CRISPR/Cas9.
- ❖ The optimized CRISPR/Cas9 system achieved single, double, and triple gene-knockout efficiencies of 97%, 90%, and 47%, respectively.

Significance

This study reports the first CRISPR/Cas9-based system for gene disruption in *I. orientalis*, which will pave the way for genome and metabolic engineering of this yeast strain for production of bioproducts.



Schematic showing assembly of CRISPR/Cas9 plasmid for multiplexed gene deletions