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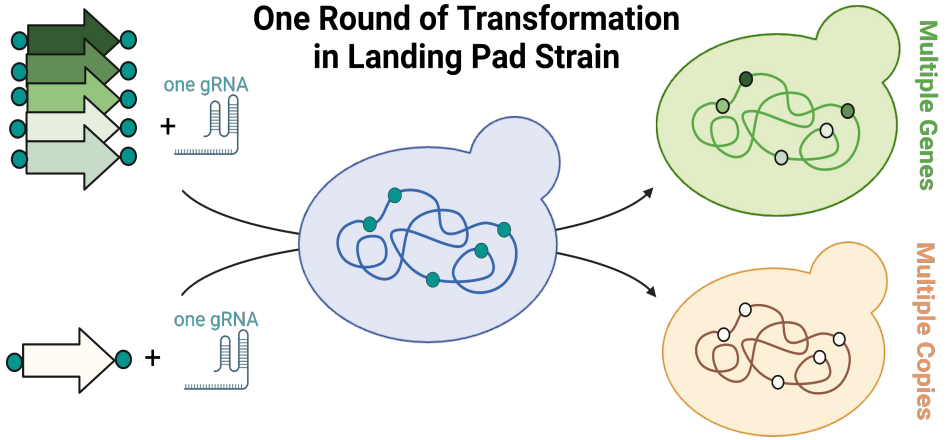
A Landing Pad System for Multicopy Gene Integration in Issatchenkia orientalis

Background/Objective

Non-conventional yeast *Issatchenkia orientalis* can grow under highly acidic conditions and, therefore, has gained interest in producing organic acids using various carbon sources. However, metabolic engineering efforts are hampered by the lack of efficient multicopy integration tools. In this work, we developed a bioinformatics pipeline to identify integration sites and a landing pad system to facilitate the construction of large, complex metabolic pathways using CRISPR/Cas9-mediated multiplex genome editing.

Approach

- Developed a bioinformatics pipeline to identify and prioritize genome-wide intergenic integration sites and screened the sites for guide RNA (gRNA) cutting efficiency, gene cassette integration efficiency, resulting cellular fitness, and GFP expression level,
- Constructed a landing pad system to enable the integration of multiple genes or multicopy of a gene in one round of transformation,
- Demonstrated the system in *I. orientalis* by integrating multiple copies of 5-aminolevulinic acid synthase to produce 5-aminolevulinic acid (5-ALA) and a biosynthetic pathway to produce succinic acid using a single gRNA.



The landing pad system can integrate multiple genes or multicopy of a gene using a single gRNA in one round of transformation.

Results

We identified 21 gRNAs in 14 loci suitable for efficient expression of heterologous genes. We also demonstrated the ability to integrate ultralong DNA (18 kb) with 80% efficiency. Finally, demonstrating the system in *I. orientalis* showed a linear increase in 5-ALA production and 9 g/L of succinic acid production from strains constructed in a single transformation.

Significance/Impacts

The landing pad system overcomes the time-consuming, laborious efforts required to integrate multiple genes or copies in metabolic engineering.

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