CABBI
CENTER FOR ADVANCED BIOENERGY AND BIOPRODUCTS INNOVATION

RT-EZ: A Golden Gate Assembly Toolkit for Streamlined Genetic Engineering of *Rhodotorula toruloides*

Background/Objective

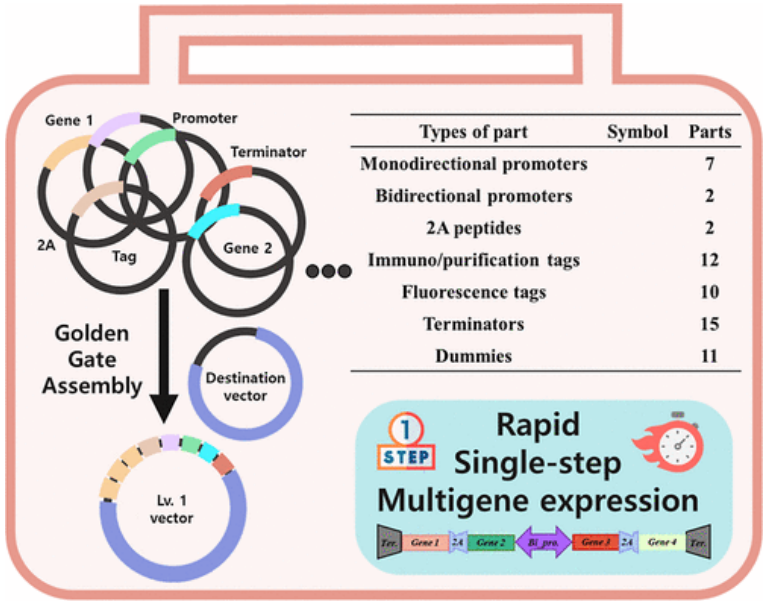
For economic and sustainable biomanufacturing, the oleaginous yeast *R. toruloides* has emerged as a promising platform for producing biofuels and other valuable chemicals. However, its genetic manipulation has been limited by its high GC content and the lack of a replicating plasmid, necessitating gene integration. To address these challenges, we developed the RT-EZ (***R***. ***t***oruloides **E**fficient **Z**ipper) toolkit, a versatile tool based on Golden Gate assembly, designed to streamline *R. toruloides* engineering with improved efficiency and flexibility.

Approach

The RT-EZ toolkit simplifies vector construction by incorporating new features such as bidirectional promoters and 2A peptides, color-based screening using RFP, and sequences optimized for both Agrobacterium tumefaciens-mediated transformation (ATMT) and easy linearization, enabling straightforward selection and transformation. A series of vectors were tested in *R. toruloides* to validate the Golden Gate assembly, promoter functionality, and fluorescent protein expression. The multigene expression was validated using 2A peptides.

Results

Validation experiments demonstrated the toolkit's functionality through successful fluorescent protein expression using mono- and bidirectional promoters, as well as effective multigene expression using 2A peptides. Notably, it can construct an expression cassette with four different genes in one assembly reaction, significantly improving vector construction speed and efficiency. The toolkit's capability was further showcased by the biosynthesis of arachidonic acid, achieved through the efficient coexpression of four pathway enzymes.



Overview of the RT-EZ toolkit.

Significance/Impacts

This toolkit provides a streamlined method for addressing genetic engineering challenges in *R. toruloides*, reducing the time and effort required for complex metabolic engineering projects and unlocking the full biotechnological potential of *R. toruloides*.

Koh et al. 2025. "RT-EZ: A Golden Gate Assembly Toolkit for Streamlined Genetic Engineering of *Rhodotorula toruloides*." *ACS Synthetic Biology*. DOI: 10.1021/acssynbio.4c00848.