A molecular genetic toolbox for Yarrowia lipolytica

**Background:** Yarrowia lipolytica is an ascomycete yeast used in biotechnological research for its abilities to secrete high concentrations of proteins and accumulate lipids. Genetic tools have been made in a variety of backgrounds with varying similarity to a comprehensively sequenced strain. Results: We have developed a set of genetic and molecular tools in order to expand capabilities of Y. lipolytica for both biological research and industrial bioengineering applications. In this work, we generated a set of isogenic auxotrophic strains with decreased non-homologous end joining for targeted DNA incorporation. Genome sequencing, assembly, and annotation of this genetic background uncovers previously unidentified genes in Y. lipolytica. To complement these strains, we constructed plasmids with Y. lipolytica-optimized superfolder GFP for targeted overexpression and fluorescent tagging. We used these tools to build the "Yarrowia lipolytica Cell Atlas," a collection of strains with endogenous fluorescently tagged organelles in the same genetic background, in order to define organelle morphology in live cells. Conclusions: These molecular and isogenetic tools are useful for live assessment of organelle-specific protein expression, and for localization of lipid biosynthetic enzymes or other proteins in Y. lipolytica. This work provides the Yarrowia community with tools for cell biology and metabolism research in Y. lipolytica for further development of biofuels and natural products.

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