Easy-to-use vectors for CRISPR genome editing in *Saccharomyces cerevisiae*: from Cas9 to MAD7

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Abstract:

CRISPR-Cas9 is currently the gold standard for genome editing in *Saccharomyces cerevisiae*. Regretfully, plasmid-based systems that allow the easy insertion of user-defined gRNAs are still lacking. The standard techniques are usually based on restriction and ligation or on PCR amplification of the whole plasmid backbone. The latter method frequently results in an unpractical two-plasmids system, in order to avoid the amplification of the long Cas9 cassette. Additionally, many traditional systems rely on auxotrophic markers, which cannot be exploited in industry-ready prototrophic strains.

In this work we built an easy-to-use single-plasmid CRISPR/Cas9 system in which the gRNA is inserted by Golden Gate Assembly, with an immediate red/white screening and assembly efficiencies of about 100%. The plasmid bears a dominant marker, to overcome strain background limitations, and allows precision editing with high efficiency. The total turnover time from parts assembly to the engineered strain is of about two weeks.

Furthermore, we used a similar approach to build and test a CRISPR/MAD7 system. MAD7 is an alternative to the widespread Cas9 nuclease, released by Inscripta Inc. under a non-exclusive licensing program for both academic and commercial uses. Thanks to the flexibility of the strategy, many combinations of parts will be tested to identify the optimal design for the nuclease expression and the crRNA structure.