Research Tools

High-Throughput Genome Editing and Engineering of Industrial Yeast, Other Fungi

View U.S. Patent No. 9,879,270 in PDF format.

WARF: P140240US03

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The Wisconsin Alumni Research Foundation (WARF) is seeking commercial partners interested in developing a new genome editing system that may be 1,000 times more efficient than other methods for modifying yeast strains critical to the brewing and biofuels industry.

Overview

As the industrial uses of yeast and other fungi expand, new systems are needed to genetically optimize strains for their intended use. In particular, the ability to manipulate prototrophic and diploid strains is critical for applications in the brewing and biofuels industry where the strains favored by lab researchers are seldom used.

Efficient genome editing and engineering requires targeted double-strand breaks and marker genes that are both selectable (to get the marker in) and counter-selectable (to get the marker back out). Existing technologies (e.g., CRISPR-Cas) suffer from limitations that limit throughput. These restrictions present major obstacles to engineering wild and industrial strains.

The Invention

UW–Madison researchers have developed expression cassettes that facilitate genome editing and sequence replacement in fungi at an extraordinarily high rate. Their HERP (Haploid Engineering Replacement Protocol) cassettes combine thymidine kinase (TK) enzyme with meganucleases, and permit hundreds of thousands of independent transformations to be obtained in a single experiment.

TK (from human Herpes Simplex Virus) serves as both a selectable and counter-selectable marker. Since the common ancestor of all fungi lacked the gene, the marker is likely of nearly pan-fungal utility. Relevant species should include *Saccharomyces cerevisiae*, *Saccharomyces mikatae*, *Saccharomyces kudriavzevii*, *Saccharomyces uvarum* and *Neurospora crassa*.

Applications

- Modifying yeast for industrial use
- HERP cassettes for genome editing or replacement of coding sequences in fungi (singly or in designed, natural or random pools)
- Other possible uses include
  - Targeted or combinatorial induction of rearrangements in chromosomes
  - Precise epitope tagging or fusion-protein construction
  - Precise replacement of cis-regulatory or non-coding sequences
  - Combinatorial introduction of libraries of genes or pathways via homologous recombination at the double-strand break
  - “Scarless” gene editing (where the resulting strain is nearly 100 percent identical to the original)

Key Benefits

- Enables efficient and direct modification of the organism of choice

- Supports high-throughput screens in the relevant genetic background and industrial condition
- Simplifies genome editing of diploid wild/industrial *Saccharomyces* strains and species for the first time

Stage of Development

The researchers have shown that the cassettes allow for manipulation of a genomic locus at rates approaching 1 percent of surviving cells, or approximately 1000x more efficiently than reported CRISPR-Cas9 rates in wild *Saccharomyces* species.

Additional Information

For More Information About the Inventors

- Christopher Hittinger

Tech Fields

- Research Tools : Other research tools

For current licensing status, please contact Jennifer Gottwald at jennifer@warf.org or 608-960-9854