**A New Genome Editing Tool for Use in a Variety of *Saccharomyces* Species**

One way in which microorganisms may be tailored to a particular process is by rational design and the incorporation of genetic traits that lead to improvements. Many model organisms like *Saccharomyces cerevisiae* have well developed tools for genetic manipulation however their use often does not extend to other species, even those that are closely related. Researchers at Great Lakes Bioenergy Research Center (GLBRC) developed a system for gene replacement that can be used to engineer a range of *Saccharomyces* species. Termed Haploid Engineering and Replacement Protocol (HERP), the method utilizes a viral gene encoding thymidine kinase (TK) for both positive and negative selection. *TK* is an ideal candidate for selection in yeast as this gene is largely absent in the fungal kingdom and thus strains can be modified *de novo*, without prior modification such as generation of auxotrophy. In addition to the use of *TK*, HERP cassettes contain inducible-*SCE1* endonuclease that introduces double-strand breaks into the chromosome, enhancing recombination with the replacement allele. Depending on the transformation method, allele replacement was shown to occur at a rate approaching 1%. The high rate of allele replacement in this system enables gene replacement by a population of variants. This was demonstrated by transformation of *S. cerevisiae* with pooled alleles from seven different *Saccharomyces* species, all of which were recovered after selection. Thus, the HERP method is well suited to high-throughput transformation and screening.

Moreover, the method works well for homozygous allele replacement in diploid strains provided that there is sequential insertion of the HERP cassette at the targeted locus. This is important since many fungal isolates exist as diploids in nature and can be difficult to sporulate. In the absence of haploidy, the preferred recombination event is allele repair via the homologous chromosome rather than replacement with a linear fragment. In *S. uvarum*, double allele replacements were recovered on the order of ~1 in 107, similar to the rate of double replacement in *S. cerevisiae*. The group is developing a next generation HERP system that will broaden its use to other members of the fungal kingdom, beyond the Saccharomycataceae. Given the genetic diversity that is known to exist in natural populations, the ability to genetically modify and screen such organisms for biofuel applications is an exciting advancement in bioenergy research.

**Reference:** Alexander WG, Doering DT, Hittinger CT (2014) High-efficiency genome editing and allele

replacement in prototrophic and wild strains of *Saccharomyces*. Genetics 198: 859-866.

**Contact**: Dr. N. Kent Peters, SC-23.2, (301) 903-5549