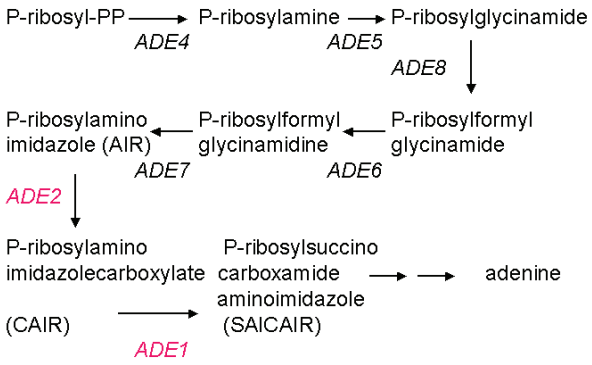
DATASHEET | Cas-CLOVER For Industrial Biotechnology

Demeetra

Cas-CLOVER: Creating High-performing Yeast Strains For Industrial Utilization

Currently, yeasts play an important role as the vector for bioproduction of biofuels, food additives and proteins due to their exceptional natural characteristics. While Saccharomyces cerevisiae has been widely used in research and bioprocessing, non-conventional yeasts, such as *Scheffersomyces (Pichia) stipitis, Komagataella phaffii (Pichia pastoris), Ogataea (Hansenula) polymorpha, Kluyveromyces lactis, Yarrowia lipolytica, Kluyveromyces marxianus* and *Ogataea thermomethanolica* are also beneficial as each has specific characteristics; thermo-tolerance, efficient protein production, lactose metabolization, just to name a few.

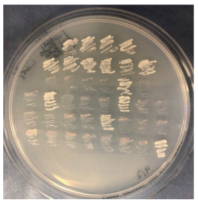
These advantageous characteristics can be exploited using gene editing technology. Cas-CLOVER technology has been shown to disrupt a pathway in yeast resulting in the accumulation of red pigment in vacuole by targeting the phosphoribosylaminoimidazole carboxylase ADE2 (a gene that catalyses the biosynthesis of purine nucleotides) locus.



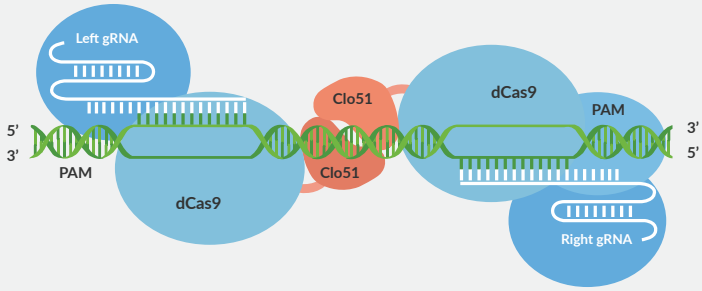
Specifically, Demeetra examined different sets of gRNA and Cas-CLOVER systems resulting with up to 90% targeted knockout efficiency.

		KO Efficiency
Clones 1-10	(1-CasCLOVER)	0%
Clones 11-20	(2-CasCLOVER)	80%
Clones 21-30	(3-CasCLOVER)	80%
Clones 31-45	(4-CasCLOVER)	90%

CM-Adenine



The Cas-CLOVER gene editing system utilizes a catalytically inactive Cas9 protein fused to the Clo51 nucleus domain which works as monomers recruited by a pair of guide RNAs (gRNA) to introduce targeted mutations. And when both subunits are properly recruited to the target-site, it leads to dimerization and activation of the Clo51 nucleus domain, leading to targeted gene disruptions.



Typically, gene downregulation is desired in order to enhance traits to increase production. Recently, an interesting study aimed to develop a method that would allow for a multiplex of upregulation and downregulation of several genes by using the advantages of gene editing which would allow for the best of many vs. one specific gene. This method could accelerate metabolic engineering efforts for the construction of future industrial systems through simultaneous manipulation of several metabolic targets especially when done with a high fidelity system like Cas-CLOVER, where off-targets won't mask or select out important traits. (2).

- Cas-CLOVER is exclusively licensed technology and sub-licensable by Demeetra
- Cas-CLOVER is functionally similar to other CRISPR technologies, but uses a different nuclease protein called Clo51, which is covered under a set of patents distinct from other CRISPR technologies
- Cas-CLOVER gene editing technology has undetectable off-target activity
- Cas-CLOVER achieves greater specificity through utilization of two guide mRNAs as well as a nuclease activity that requires dimerization of subunits associated with each guide RNA
- No known IP entanglements or legal uncertainty that may exist with other gene editing technologies (i.e. CRISPR)

The future of Cas-CLOVER is exciting for industrial biotechnology as it provides numerous opportunities. Demeetra is offering clear commercial freedom to operate and simple accessible licenses to commercial users. We are interested in special collaborations with academic groups that can result in methods to increase production in yeast which can ultimately be adopted commercially to enhance biofuels, food additives and proteins.

1. Cai, P., Gao, J., & Zhou, Y. (2019). CRISPR-mediated genome editing in non-conventional yeasts for biotechnological applications. Microbial Cell Factories, 18(1), 1–12. https://doi.org/10.1186/s12934-019-1112-2
2. Kildegaard, K. R., Tramontin, L. R. R., Chekina, K., Li, M., Goedecke, T. J., Kristensen, M., & Borodina, I. (2019). CRISPR/Cas9-RNA interference system for combinatorial metabolic engineering of Saccharomyces cerevisiae. Yeast, 36(5), 237–247. https://doi.org/10.1002/yea.3390