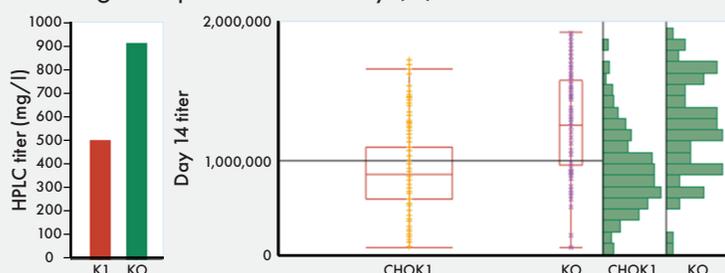




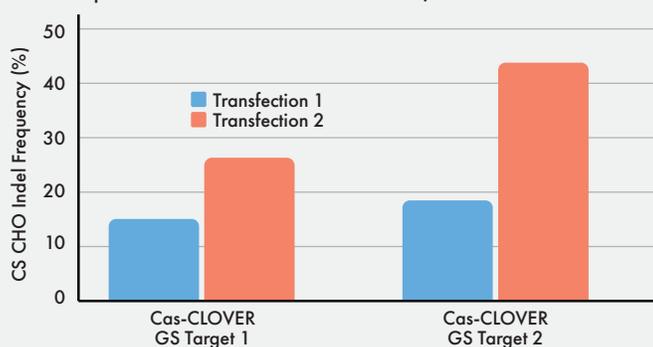
Cas-CLOVER: Enhanced Expression Systems To Improve Bioprocessing

In order to produce large quantities of life saving biotherapeutics, cutting costs and saving time where possible, while at the same time not decreasing quality, is critical. When it comes to bioprocessing, scientists have found that modifying current mammalian expression platforms where Chinese hamster ovary (CHO) cells are most commonly used is the answer to increasing efficiency.

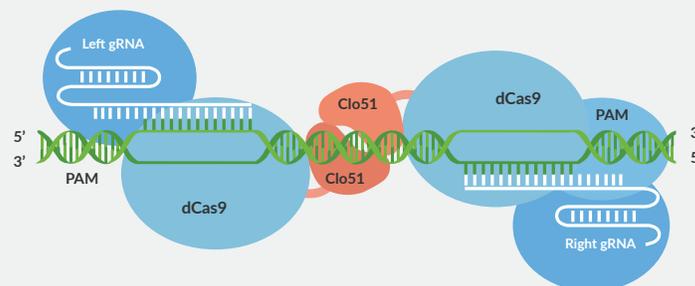
CHO cells can be optimized through advanced gene editing. For example, when the endogenous glutamine synthetase (GS) gene is knocked out in CHO cells, a six-fold increase in high-producing cell lines is achieved. Combining newer gene editing tools that have specific targeting capabilities with the robust CHO expression systems has significantly impacted bulk cell culture productivity. There's an increased rate of high-producing cells resulting in improved efficiency (1).



Demeetra is introducing their Cas-CLOVER technology for commercial bioprocessing as well as newly edited GS knockout CHO cell lines. Cas-CLOVER differs from CRISPR/Cas9 in that it is a dimeric nuclease system lacking detectable off-target mutagenesis. The specificity of Cas-CLOVER enables multiple rounds of targeting at one locus to increase indel frequency without introducing the risk of unwanted off-target mutations. Shown here are indel frequencies of 18% and 43% at the CHO GS locus for one and two rounds of targeting, respectively. These on-target frequencies in CHO cells are higher than reported for ZFN and comparable to those of CRISPR/Cas9.



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.



Companies like Demeetra recognize the impact that not only gene editing tools, but GS-KO CHO cells can have on the future of science, bioprocessing, and ultimately saving lives. In particular, Demeetra provides licenses for their novel gene editing Cas-CLOVER system as well as GS-KO CHO cell lines either sold separately or together to improve research and/or large scale operations. There's no need to change current GS vector expression system with gene of interest (GOI), promoter, and selection marker. Adding your system to GS-KO CHO cells can boost efficiency.

Compared with CRISPR/Cas9, Cas-CLOVER offers...

- A cleaner gene editing alternative for commercial bioprocessing
- Similar indel frequencies
- A highly specific system showing no detectable off-targets
- Special license structures for CDMOs
- Issued patents that allow clear freedom to operate
- Evaluation licenses for Cas-CLOVER and GS CHO knockouts - separately or together - to convert into commercial licenses

Future use of gene editing systems like Cas-CLOVER is exciting for therapeutic bioprocessing as they provide various 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 productivity and efficiency while consistently bringing quality therapies to market.