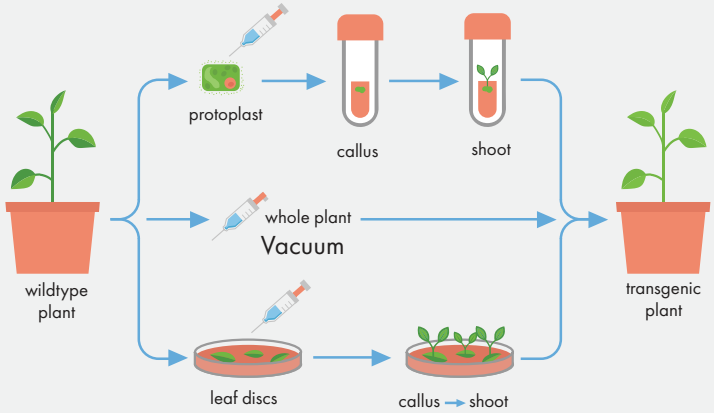
DATASHEET | Cas-CLOVER For Agriculture

Demeetra

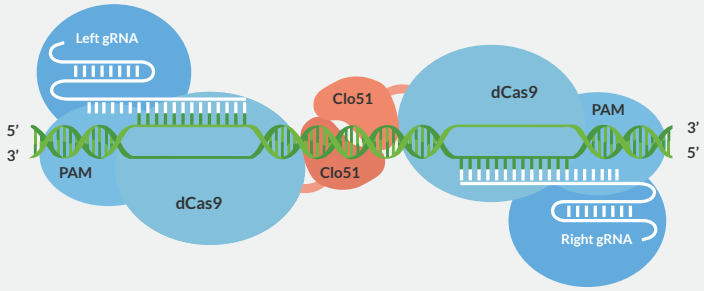
Cas-CLOVER: Increasing The Productivity In Crop Trait Engineering, The Non-GMO Way

Our rapidly growing population with threats of climate change and global pandemics continue to be factors that pressure our food and medicine security, increasing the need for more productive agriculture traits and biomanufacturing systems is essential. Traditional crop breeding techniques are challenging and remain a lengthy process. Additionally, older generation transgenic technologies produce GMO's with permanent heterologous genes inserted. Conversely, advanced gene editing technologies enable plant biotechnologists with the ability to make targeted knockouts and insertions. This scarless gene editing means no heterologous genes are left in the plant genome, producing a non-GMO crop and simplifying the process.

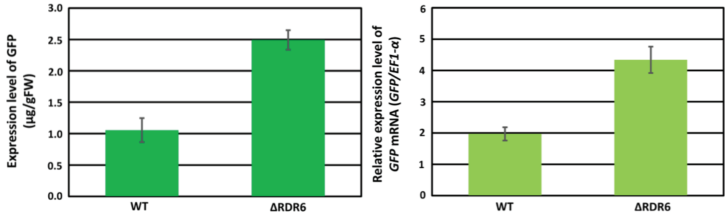
CRISPR-Cas9 technology has been validated as a very useful advanced gene editing technology on an academic level, but suffers from commercial licensing restrictions. A recent review identifies nearly 30 engineered traits for disease resistance and abiotic stress in important crops (3), most of which will never be used on a commercial level due to licensing restrictions. Like chemical mutagenesis but on a lower level, CRISPR-Cas9 introduces abundant off-target mutations, increasing the need for backcrossing the crop to remove these unwanted mutations.



Cas-CLOVER was first introduced and validated to have little to no off-targets due to its dimeric nature in cell lines such as human T-cells (1). The Cas-CLOVER gene editing system utilizes a catalytically inactive Cas9 protein fused to the Clo51 nuclease 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 nuclease domain, leading to targeted gene disruptions.



Recently, Demeetra AgBio validated the activity of Cas-CLOVER in plants by targeted inactivation of the RNA-dependent RNA polymerase 6 (RDR6) gene in tobacco (2). We achieved an efficiency of at least 18% for our first target which is significant for tobacco. Edits such as the RDR6 gene may be valuable as they can result in more robust recombinant protein production for rapid and scalable vaccine and medicine manufacturing, as shown in previous publications. In fact, the vaccine for the last Ebola outbreak was produced in transgenic tobacco; showing the value of genetic editing tools in agriculture.



- RDR6 KO reduces the plants RNA interfering system which can limit exogenous protein production
- RDR6 KO tobacco have been generated using CRISPR/Cas9
- Commercial applications are unknown for the Cas9 generated KO, likely due to licensing restrictions
- Demeetra created RDR6 knockout tobacco as a POC study for Cas-CLOVER in plants and to generate a strain with improved biologics production

The validated Cas-CLOVER activity in plants is exciting for crop trait development as it opens up 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 traits which can ultimately be adopted commercially to enhance food production while being mindful of the environment.

1. Li, X., Wang, X., Tong, M., Tan, Y., Down, J. D., Shedlock, D. J., ... Diego, S. (2019). Cas-CLOVER: A High-Fidelity Genome Editing System for Safe and Efficient Modification of Cells for Immunotherapy, 92121.
2. Matsuo, K., & Atsumi, G. (2019). CRISPR/Cas9-mediated knockout of the RDR6 gene in Nicotiana benthamiana for efficient transient expression of recombinant proteins. Planta, 250(2), 463–473. https://doi.org/10.1007/s00425-019-03180-9
3. Razzaq, A., Saleem, F., Kanwal, M., Mustafa, G., Yousaf, S., Arshad, H. M. I., ... KhanJoyia, F. A. (2019). Modern trends in plant genome editing: An inclusive review of the CRISPR/Cas9 Toolbox. International Journal of Molecular Sciences, 20(16). https://doi.org/10.3390/ijms20164045