Title: Developing detection and countermeasure tools for CRISPR-based genome engineering tools in plants

Authors: Yang Liu[1]* (Liuy4@ornl.gov), Guoliang Yuan,[1] Xiaohan Yang,[1] and **Paul E. Abraham**[1]

Institutions: [1] Bioscience Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Website URL: https://seed-sfa.ornl.gov/

Project Goals: The Secure Ecosystem Engineering and Design (SEED) Science Focus Area (SFA), led by Oak Ridge National Laboratory, combines unique resources and expertise in the biochemistry, genetics, and ecology of plant-microbe interactions with new approaches for analysis and manipulation of complex biological systems. The long-term objective is to develop a foundational understanding of how non-native microorganisms establish, spread, and impact ecosystems critical to U.S. Department of Energy missions. This knowledge will guide biosystems design for ecosystem engineering while providing the baseline understanding needed for risk assessment and decision-making.

Abstract Text:

CRISPR/Cas-based gene editing tools have been widely applied in plants. However, genome engineering in plants is currently limited by the reliability and performance of these tools. At present, typically only one or a few genes can be edited at a time and most successful edits are imprecise end-joining of double-stranded breaks (DSBs). While these knockout mutations can be useful in some contexts, these are often inadequate to confer complex traits needed to improve DOE-model biofeedstocks. More sophisticated gene edits are now becoming available with advanced tools such as based and prime editors, yet these tools have only recently been implemented in plants and successful edits are often rare events. To fully realize the potential of CRISPR tools in plants, methods are needed to assess the performance of these tools faster, with less cost, and at scale. For this reason, we developed a biosensor system for real-time detection of active CRISPR/Cas tools *in planta*[1]. Specifically, several non-functional GFP genes which cannot emit fluorescence signals together with single guide RNAs (sgRNAs) were built as biosensors. In the presence of various CRIPSR tools such as Cas9 endonuclease, base editors (BEs), and prime editors (PEs), nonfunctional GFP mutants can be successfully converted into the functional GFP gene, generating green fluorescence.

Over the past several years, there has been a rapidly growing interest in using plant viruses as vectors to deliver and spread genome editing tools. This is because the use of viruses, to create gene edits through infection, has been recognized as a method to overcome current bottlenecks in the production of gene edited plants at scale. The consequent ability to edit plants through viral infection introduces concerning biosecurity risks. Also, there are increasing concerns about the biosafety issues caused by CRISPR tools, such as off-target effects of CRISPR/Cas systems,

CRISPR-based contaminating gene drive, and potential malicious genomic modifications mediated by CRISPR tools. For these reasons, in addition to continually working to optimize biosensors for the real-time detection of active CRISPR/Cas tools[1], we are developing countermeasure systems for inhibiting the activity of CRISPR/Cas systems in planta. Using the developed biosensor systems, we successfully demonstrated that AcrIIA4 and AcrIIA5 could inhibit the activities of CRISPR/Cas9 and one base editor in Arabidopsis protoplasts. The functionality of AcrIIA4 and AcrIIA5 will be further investigated in *Nicotiana benthamiana* (tobacco) and *Populus tremula* x *P. alba* 717-1B4.

References/Publications

1. Yuan, G. L.; Hassan, M. M.; Yao, T.; Lu, H. W.; Vergara, M. M.; Labbe, J. L.; Muchero, W.; Pan, C. T.; Chen, J. G.; Tuskan, G. A.; Qi, Y. P.; Abraham, P. E.; Yang, X. H., Plant-Based Biosensors for Detecting CRISPR-Mediated Genome Engineering. *Acs Synth Biol* **2021**, 10, 12, 3600–3603

Funding Statement: The Secure Ecosystem & Engineering Design Science Focus Area is sponsored by the Genomic Science Program, U.S Department of Energy, Office of Science, Biological and Environmental Research, under FWP ERKPA17. Oak Ridge National Laboratory is managed by UT-Battelle, LLC for the U.S. Department of Energy under contract no. DE-AC05-00OR45678. This program is supported by the U. S. Department of Energy, Office of Science, through the Genomic Science Program, Office of Biological and Environmental Research, under FWP ERKP123.