

## **Real-time molecular detection systems for CRISPR-Cas genome engineering tools**

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**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:** CRISPR/Cas-based genome-editing has recently emerged as a powerful technology for genetic modifications in various organisms for a wide range of applications. Different CRISPR-Cas genome engineering tools, such as Cas9 nucleases, base editors, and prime editors, have been developed and widely used in ‘loss-of-function’ and ‘gain-of-function’ studies. Meanwhile, there are concerns about the risks associated with potential unwanted and unintended DNA changes that might accidentally arise from CRISPR-mediated gene editing. Potential off-target effects are currently unavoidable and very difficult to detect, requiring whole-genome sequencing for thorough evaluation, which is time consuming and costly. Therefore, it is critical to develop biosensor technologies for detecting the activities of CRISPR-Cas gene editing systems. Here, we aim to develop real-time detection systems that can indicate the presence of functional gene-editing systems for gene knockout, base editing, and prime editing in plants. To achieve this goal, we have created fluorescence-based molecular biosensors that are able to effectively detect (through fluorescence microscope) knockout, base editing and prime editing using protoplast transient expression and *Agrobacterium*-mediated leaf infiltration. Also, we are working on incorporating two visible reporters for real-time, noninvasive detection of Cas9 nucleases, base editors, and prime editors in plants without need for tedious analysis under fluorescence microscope. One of the reporters can indicate genome engineering under UV light. The other reporter can be visualized by naked eyes without the need of using special equipment or chemical treatments. These biosensors with visible reporters will not only simplify the detection procedures, but also be directly integrated with high-throughput plant phenotyping facility to link genes to traits.