Title: Automated high-throughput genome editing of TAG-related genes in plants

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Project Goals: This project will closely link the Conversion and Feedstock themes by integrating techniques developed by the Conversion theme, namely high-throughput automation, with a major goal of the Feedstock theme: maximizing TAG output in plants using CRISPR genome editing.

Abstract Text: Developing transgenic or genome-edited plants is a time- and labor-intensive process. However, iBioFAB offers a potential solution through high-throughput robotic automation of plant genome editing. In our project, we are developing automated high-throughput processes for CRISPR genome editing of triacylglyceride (TAG) content in three plant protoplast systems: *Nicotiana benthamiana*, Maize B73, and Sorghum TX623. We first established protocols for isolation and transfection of protoplasts from these three plant systems with high efficiency. We then constructed CRISPR deletion vectors targeting two TAG-related and three photosynthesis genes to be used as proof of concepts for automated genome editing in plants. Furthermore, we have programmed the robotic protocols enabling automated protoplast transfection. In the coming months, we will develop robotic protocols for automated protoplast isolation and TAG quantification using fluorescent staining of lipids. Finally, we will employ the full high-throughput system to target a variety of TAG-related genes and determine their individual and combinatorial affects on TAG accumulation. Our work will enable rapid and accurate determination of the effects of specific genome edits in plants, which can later be applied to developing germline-edited plant varieties.

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