CABBI
CENTER FOR ADVANCED BIOENERGY AND BIOPRODUCTS INNOVATION

Enhancing Lipid Production in Plant Cells through Automated High-throughput Genome Editing and Phenotyping

Background/Objective

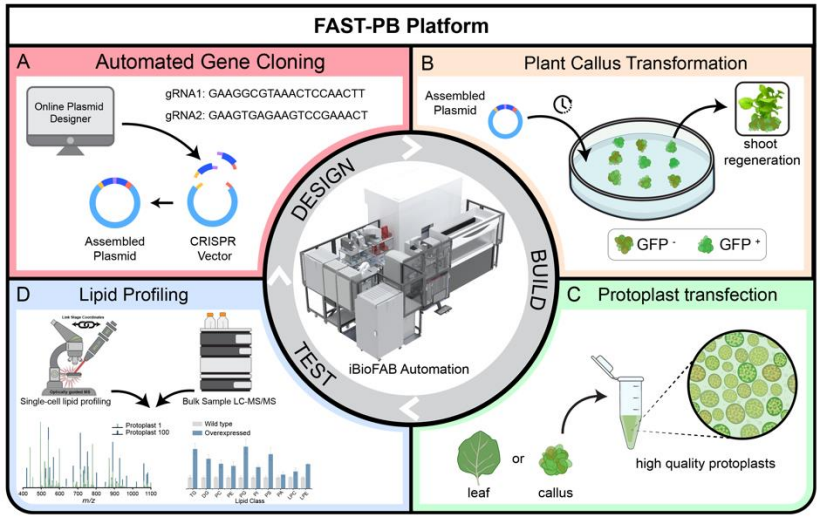
Plant bioengineering is a time-consuming and labor-intensive process with no guarantee of achieving desired traits. To overcome this limitation, we sought to integrate an automated biofoundry with single-cell metabolomics to expedite the engineering of plant genomes and characterization of cellular effects, which has never been done before. Here, we present a fast, automated, scalable, high-throughput pipeline for plant bioengineering (FAST-PB) in model systems.

Approach

This pipeline seamlessly integrates the iBioFAB biofoundry for automated synthetic biology with matrix-assisted laser desorption/ionization Fourier transform ion cyclotron resonance mass spectrometry (MALDI FT-ICR MS) for high-throughput single-cell lipid identification.

Results

Using FAST-PB in protoplasts, we found that 49 PEG2050 increased transfection efficiency by over 45%. For proof-of-concept, we established a reporter-gene-free method for CRISPR editing and phenotyping via mutation of *high chlorophyl fluorescence 136*. Diverse lipids were enhanced up to sixfold using CRISPR activation of lipid controlling genes. Lastly, FAST-PB enabled high-throughput single-cell lipid profiling by integrating MALDI-MS with the biofoundry, differentiating engineered and unengineered protoplast and callus cells using single-cell lipidomics.



Overview of the FAST-PB for high-throughput genome editing and lipid 872 engineering in protoplast and callus cell systems.

Significance/Impacts

These innovations massively increase the throughput of synthetic biology, genome editing, and metabolic engineering and may change what is possible using single-cell metabolomics in plants, such as C_4 grasses. This workflow streamlines discovery, characterization, and fine-tuning of traits in highly scalable small cell cultures, leading to the regeneration of full plants after the desired phenotypes have been optimized.

Dong et al. 2025. "Enhancing lipid production in plant cells through automated high-throughput genome editing and phenotyping." *The Plant Cell*. DOI: 10.1093/plcell/koaf026.