Gene stacking approaches to built and control metabolic pathways in plants

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Engineering plants with complex metabolic pathways or multiple traits is often inhibited by the number of genes that are required to reach the final product. It also built the need of synthetic biology tools to express multiple genes with controllable expression strengths and in specific tissues. Although there already exists multiple methods for gene stacking into plant binary vectors (traditional, BioBrick, Golden Gate, Gateway, Gibson cloning, etc.), we will present a strategy using a combination of tools including *in vitro* and *in vivo* assembly methods to stack and control the expression of multiple gene cassettes (promoter::ORF::terminator). To facilitate the upstream assembly of functional gene cassettes and regulatory tools, we have also developed a library of Golden Gate cloning-compatible promoters, ORF, and terminators as well as novel expression tools. Importantly, the assembly of these functional gene parts is not limited to Golden Gate assembly, providing the scientist the flexibility to choose whatever method suits their needs best. Ultimately, functional gene cassettes are stacked using yeast assembly based on overlapping sequences, enabling homologous recombination. *In vivo* yeast assembly has been utilized to generate entirely synthetic chromosomes and genomes, and thus, we believe the development of these tools have the potential to aid scientists and engineers looking at stacking multiple genes to entire regions of chromosomes.

Funding statement
This work was part of the DOE Early Career Award and the DOE Joint BioEnergy Institute (http://www.jbei.org) supported by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U.S. Department of Energy.