A robust gene stacking method utilizing yeast assembly for plant synthetic biology

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Project Goals:

Plant synthetic biology efforts have been hampered by a dearth of versatile transformation vectors, DNA part libraries, and efficient assembly strategies. Here, we present a strategy utilizing *in vivo* yeast homologous recombination to assemble multiple gene cassettes to facilitate plant metabolic engineering, which we have named jSTACK. In doing so, we have also generated a library of DNA parts consisting of promoters, genes, and terminators that will be publicly available as a resource to the plant synthetic biology community. We demonstrate how this method can facilitate pathway engineering of molecules of pharmaceutical interest, production of potential biofuels, and shuffling of disease resistance traits between crop species. *In vivo* homologous recombination has been leveraged for the large-scale DNA assembly of synthetic chromosomes and genomes in microbes. Likewise, our approach extends this technology to plants, providing a powerful alternative to conventional approaches for stacking traits and genes to address many impending agricultural challenges.

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