

140. Stacking of Biomass Traits

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Project Goals: Our focus is on the development of plants with biomass optimized for downstream processing into biofuels.

Engineering of plants with a composition of lignocellulosic biomass that is more suitable for downstream processing is of high interest for next-generation biofuel production. Lignocellulosic biomass contains a high proportion of pentose residues, which are more difficult to convert into fuels than hexoses. Therefore, increasing the hexose/pentose ratio in biomass is one approach for biomass improvement. A genetic engineering approach was used to investigate whether the amount of pectic galactan can be specifically increased in cell walls of Arabidopsis fiber cells, which in turn could provide a potential source of readily fermentable galactose. Stacking of AtUGE2 and GalS1 genes indicates that their simultaneous overexpression increases the cell wall galactose to much higher levels than can be achieved by overexpressing either one of these proteins alone. Engineering plants with complex metabolic pathways or multiple traits is challenging because of the number of introduced genes that are required to reach the final product. Therefore, there is a great need for synthetic biology tools to express multiple genes (gene stacking) with controllable expression strengths and in specific tissues. We present a strategy utilizing in vivo yeast homologous recombination to assemble multiple functional gene cassettes (promoter::ORF::terminator) together. To facilitate the upstream assembly of functional gene cassettes, we have developed a library of Golden Gate cloning-compatible promoters, ORFs, and terminators. Importantly, the assembly of these functional gene parts is not limited to Golden Gate assembly, providing scientist the flexibility to choose whatever method best suits their needs. Ultimately, functional gene cassettes are stacked using yeast assembly based on overlapping terminator sequences, enabling homologous recombination. Using this newly developed jStack method, we now aim to further improve the galactan engineering system and in addition combine the increase in galactan with a decrease in lignin. Furthermore, we aim to stack genes in order to engineer plants with increased galactan deposition in the roots, thus improving interactions between plants and beneficial microbes.

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