

Modular Assembly of Gene Constructs for Engineering Lipid Accumulation into Energycane

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Project goals

The Renewable Oil Generated with Ultra-productive Energycane (ROGUE) project aims to engineer the two most productive American biofuel crops, energycane and *Miscanthus*, to produce a sustainable supply of biodiesel, biojet fuel and bioproducts. The main aim of this work is:

- 1) Adding value to energycane for production of advanced biofuels.**
- 2) Converting energycane into a lipid producing crop while retaining its superior biomass accumulation.**

Abstract

Metabolic engineering to divert carbon flux from sucrose to oil in a high biomass crop like energycane has been proposed as a strategy to boost lipid yields per acre for biodiesel production (Zale et al. 2016). The energy content of plant oils in the form of triacylglycerols (TAGs) is two-fold greater compared to carbohydrates. However, vegetative plant tissues do not accumulate oil to a significant amount since fatty acid synthesis in these tissues serves primarily membrane construction, in addition TAGs undergo rapid turnover. Therefore, our objectives include:

- 1) Increasing fatty acid synthesis by expressing a transcription activator of fatty acid biosynthetic genes,
- 2) Increasing TAG synthesis from diacyl-glycerol and acyl-CoA by over-expression of rate limiting enzymes,
- 3) Optimizing TAG storage by limiting the access of lipases to TAG storage compartments.

Experiment

To explore the effect of different versions of target genes under different regulatory signals on TAG accumulation in vegetative tissue we developed a library of regulatory elements and open reading frames. These components were used for modular assembly into multi-gene constructs by Golden Gate cloning. Gene expression cassettes were co-delivered with the selectable *nptII* expression cassette by biolistic gene transfer into energycane callus. Plants are currently regenerating on geneticin containing culture medium and will be analyzed for presence and expression of target constructs by PCR and quantitative RT-PCR, respectively. Plants will also be analyzed for TAG content by Gas-Chromatography and Mass Spectrometry (GC-MS).

References

Zale, J., J.H. Jung, J.Y. Kim, B. Pathak, R. Karan, H. Liu, X. Chen, H. Wu, J. Candreva, Z. Zhai, J. Shanklin, F. Altpeter. 2016. Metabolic engineering of sugarcane to accumulate energy-dense triacylglycerols in vegetative biomass. *Plant Biotech. J.* 14: 661-669.

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