

## **257b New Processing Traits for Plant-Based Production of Fuels and Chemicals**

*R. Michael Raab, Kyle L. Jensen, Jeremy C. Johnson, Karl Ruping, and Humberto De la Vega*

Agrivida is developing a suite of biotechnologies that enable the economic conversion of plants into fermentable sugars, which can be subsequently converted into a variety of fuels and chemicals. Among the technologies under development, our GreenGenes™ enzymes help consolidate the saccharification process within the plant itself. By enabling high-titer expression of the necessary processing enzymes by the plant, it is possible to eliminate the requirement for exogenous enzyme addition during saccharification, resulting in significant savings for manufacturers.

Previous attempts to produce processing enzymes in plants have been problematic, in part because of the effect of the expressed enzymes on plant physiology. We have developed intein-modified versions of  $\alpha$ -amylase that have substantially decreased activity at ambient conditions. Inteins are self-splicing protein fragments that can eliminate enzyme activity when placed within a parent protein. This activity can be recovered upon intein splicing, whose stimulus is amenable to engineering approaches and can be designed to coincide with existing processing conditions. Here we show the results of our *in vitro* development work and demonstrate the modulation of amylase activity by intein modification.