Optimizing RNA *in situ* Hybridization for Stem Parenchyma Cell-specific Promoter Characterization in Energycane

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Project Goals:
Renewable Oil Generated with Ultra-productive Energycanes—or ROGUE—is engineering the two most productive American crops—energycane and Miscanthus—to produce a sustainable supply of biodiesel, biojet fuel, and bioproducts. This work investigates mature stem parenchyma cell-specific genes with the aim to increase oil production in the stem.

Abstract:
Triacylglycerols (TAG) are major components of plant oil. Engineering energycane to produce an abundance of TAG for further conversion into biodiesel, biojet fuel, and bioproducts, is an important goal of ROGUE. Constitutively engineering TAG biosynthesis throughout the plant may produce pleiotropic effects, therefore manipulations should be restricted in mature stem parenchyma cells, where an efficient conversion of stored photoassimilates into TAG is practicable. However, the information of mature stem parenchyma cell-specific genes still lacks up to now. RNA *in situ* hybridization is a powerful tool to determine gene tissue specific expression at the cellular level. The lack of the previous report in energycane and the hard texture of mature stem hinders the histological analysis using RNA *in situ* hybridization. Here, we presented an optimized method for RNA *in situ* hybridization in energycane. Eight-micron thick paraplast-embedded cross sections can be achieved for both immature stem (internode 5) and mature stem (internode 16). This optimized method has been validated by hybridizing with an anti-sense probe against a *ScLSG* gene, which has been reported to express in the stem. Now, the method in energycane works consistently. We will be using this method to screen for the best candidate gene that will have higher abundance in pith parenchyma cells of the mature stem. Once the mature stem parenchyma cell-specific promoter is being identified, it will be used for engineering TAG biosynthesis in energycane.

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