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GP-WRITE PILOT PROJECT PROPOSAL

Name of Project: Installing "mini-symplastomes" in crops

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Background: There is a fledgling effort in our labs to design, synthesize, and install the first (that we know of) synthetic chloroplast genome "synplastome™" in tobacco (funded by ARPA-E) and potato (funded by DARPA). These species have plastomes of approximately 150 kb in multiple copies that reside in each of the dozens of chloroplasts and other plastids within each plant cell. The synplastome concept is a compelling entry point into synthetic genome design and implementation in plants. The plastome is relatively compact and plastomes have conserved structures among individuals and even plant species.

Technical Idea: We are facing some current issues in the *in vitro* assembly of the synplastome, and hereby propose to resynthesize and introduce "mini-symplastomes" as a workaround to the assembly and insertion of a single 150 kb molecule into the chloroplast. The mini-symplastome concept would use fluorescent proteins and selectable markers on each of the three-to-four mini-symplastomes that would be introduced together into chloroplasts in attempt to replace the endogenous plastome via *in vitro* antibiotic selection. There is precedence in nature for such a concept to be functional: dinoflagellates (a type of eukaryotic marine plankton) do not have a single chloroplast genome. Their chloroplast genes exist in "minicircles" (Howe et al. J Exp Bot 2008; 59: 1035-45). Thus, we propose to use the dinoflagellate bioinspiration to solve a practical problem in the first crop synthetic genome application. Additional funding for DNA synthesis and potential collaboration with other eukaryotic synthetic genomics experts could make a significant difference in the success of introducing symplastomes into crops.

Utility: Transgenes expressed via the plastome can have very high levels of expression, but current technology limits the number of genes that can be introduced. The plastome is an attractive vehicle for metabolic engineering in plants given the ability for relatively simple coordinated expression and introduction of genes in a single event. Symplastomes or mini-symplastomes could be used to endow complex metabolic traits into crops, such as improved photosynthesis and nitrogen fixation, traits that represent disruptive technologies to greatly enhance sustainability of crop agriculture. Since chloroplasts are maternally inherited in most plant species, there would be a degree of built-in bioconfinement of genetically engineered DNA; i.e., it would not be expected to be widely spread via pollen.

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"Fit" For GP-write: This pilot project would be a key 'plus-up' of one of the very first synthetic genomics projects in crop plants.