

## Enabling the RNA revolution; Cell-free dsRNA production and control of Colorado potato beetle

RNA interference holds great potential as a new non-transgenic tool for crop protection and trait improvement because of its ability to very selectively downregulate gene expression in many different organisms, such as insects, fungi, plants, nematodes, and viruses. RNAi via transgenic delivery has been successfully used to protect crops from viruses and to introduce desirable traits. Recent examples include SmartStax® PRO (corn rootworm protection), Vistive® Gold (modified oil), Innate® potato (reduced browning/acrylamide) and Rainbow Papaya (viral protection). However, large-scale and cost-effective production of dsRNA for non-transgenic delivery remains a challenge. Currently, dsRNA molecules are primarily synthesized through *in vitro* transcription (IVT) and fermentation both of which are costly and have inherently limited scalability hindering the commercial viability of dsRNA crop protection. At GreenLight Biosciences, we have developed a cell-free RNA production platform that overcomes the historic challenges of purity, cost, and rapid scale-up performance found with conventional RNA synthesis. Our platform delivers the same bioactivity and specificity as IVT-based dsRNA with a projected cost of <\$0.50/g dsRNA and scalability to metric tons. Our cell-free platform breaks down RNA from a low-cost polymeric RNA-containing substrate such as yeast into nucleoside monophosphate (NMP) building blocks and rebuilds these into the RNA product of interest using proprietary enzymes, a DNA template, and a low-cost energy source. The only component that varies in the production process from one RNA product to another is the corresponding DNA template. Thus, when novel targets are discovered, the platform can easily generate the corresponding dsRNA products at scale by simply preparing a new DNA template. We have shown our process to produce highly bioactive dsRNA with our 2018 and 2019 field trials for our upcoming product for Colorado Potato Beetle dsRNA insect control. We are currently expanding our Crop Protection portfolio to other insects, fungi and plant viruses.