Implementing Recombineering and Viral Vectors for Enhanced Homologous Recombination-Based Plant Genome Editing

Matthew Neubauer

The predominant methods of plant genetic engineering rely on double-stranded DNA (dsDNA) breaks to be made in the genome and subsequently repaired. In plants, dsDNA breaks are typically repaired by non-homologous end joining, rather than the more precise method of homologous recombination (HR). My work aims to address this limitation by implementing lambda Red recombineering to enhance the rate of HR in plants. Recombineering is a genetic engineering technique that has been used for decades to edit large pieces of DNA in prokaryotes. Originally derived from the lambda prophage, the system consists of three major components, Exo, Beta, and Gam, which are capable of mediating HR between a provided repair template (RT) and actively replicating DNA. I have established a system to deliver the lambda Red machinery to plant tissue and detect successful homologous recombination events in N. benthamiana. To deliver a recombineering-compatible RT to plant tissues, I am utilizing geminivirus-based viral vectors. These viral vectors can replicate inside plant cells, enhancing the amount of RT available for HR. To optimize the system, I have generated a new viral vector system based on the Beat Curly Top Virus (BCTV). These new viral vectors are compatible with the GoldenGate cloning method GoldenBraid, making it easy to test different cargoes, promoters, and viral genes. In addition to enabling high levels of gene expression in plant cells, I have demonstrated that these new BCTV-based vectors can successfully deliver RT to N. benthamiana for HR-based gene editing. Future work aims to optimize the expression of the recombineering proteins in plants to enhance the rate of HR in plant cells.