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Transposon mutagenesis in *Glycine max*: generating mutant alleles in soybean

Ed McAssey,* Center for Applied Genetic Technologies, University of Georgia, Georgia, USA

Lauren Lail, Center for Applied Genetic Technologies, University of Georgia, Georgia, USA

C. Nathan Hancock, Department of Biology and Geology, University of South Carolina, South Carolina, USA

Wayne Parrott, Institute of Plant Breeding, Genetics and Genomics, University of Georgia, Georgia, USA

While sequencing projects have identified the number, location, and expression levels of many genes in soybean, the function of the majority of these genes remain unknown. Additionally, the fact that soybean is a paleopolyploid means that duplicate genes can hinder the ability of a single gene knock-out to confer a mutant phenotype. To address both of these points, mutant alleles across the soybean genome are being developed using transposon mutagenesis with the rice transposon *mPing*. In parallel, new transposon-tagging vectors are being developed, characterized, and deployed, including an activation tag to create dominant phenotypes as well as an insertional tag. Finally, tissue culture is being used to increase the insertion frequency of plants with first-generation *mPing* transposon constructs. Since the location of *mPing* insertions are known in a subset of our pre-tissue culture samples, we are monitoring their excision using PCR. For pre-tissue culture lines with unknown *mPing* locations we are using transposon display to compare pre- and post-tissue culture samples for evidence of activation. So far, the PCR based assay appears to show considerable excision from known sites in tissue culture samples. A sequencing library construction protocol uses outward facing *mPing* primers with Illumina overhangs to simultaneously amplify transposon-soybean DNA junctions and add Illumina sequence necessary for flow cell binding. Additionally, we are exploring the possibility of using whole genome sequencing to rapidly identify transposon locations in the genome. A key advantage of whole genome sequence is that both transgenic and native TEs can be simultaneously genotyped. Subsequently, a bioinformatics pipeline maps reads to the soybean genome to identify the locations of new insertions and prepares the information for upload to Soybase.org.