

35. Next Generation Protein Interactomes for Plant Systems Biology and Biomass Feedstocks Research

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Project Goals: Elucidate interactome characteristics giving rise to desirable phenotypic traits in biofuel feedstock crops by rapidly identifying all their protein-protein interactions using the en masse next-gen yeast two-hybrid screening system, ProCREate.

In order to keep up with global energy demands, it is imperative we acquire more knowledge of biofuel feedstocks for improving their cultivation and energy yield. Knowledge of proteome- wide protein-protein interaction (PPI) networks, or interactomes, that promote robust plant growth or that are perturbed by pathogens could progress strategies for improving cultivation. However, current technologies for obtaining interactome data are not suitable for non-model plants like switchgrass or sorghum because of time, cost, and sensitivity constraints. Even the largest high quality PPI map for the model plant *Arabidopsis thaliana* (*Arabidopsis* Interactome 1 or AI-1) contains an estimated 2% of the interactome, and took upwards of 5 years and \$8 million to finish¹. To address this problem, we have developed a yeast two-hybrid (Y2H) system, ProCREate, that can currently generate interactome data 4x faster and 30x less expensive than the Y2H ‘gold standard’ assay used to generate AI-1. ProCREate enables en masse pooling and massively paralleled sequencing for the identification of interacting proteins by exploiting Cre- lox recombination. Only interacting proteins can induce reporter gene expression of Cre Recombinase and subsequent Cre-mediated recombination of plasmids containing mutant loxP sequences. The irreversible double mutant loxP linkage of each protein’s corresponding coding sequence has allowed us to identify protein interactions using Illumina paired-end sequencing. Assay quality was measured using a set of ~3,300 *Arabidopsis* ORFs (AIrepeat) previously screened six times for estimating the sensitivity of the Y2H ‘gold standard’ assay. Combined data from three replicate ProCREate screens of libraries of ~1600 ORFs x libraries of ~2800 ORFs showed a significant increase in assay sensitivity and in sampling sensitivity compared to six replicate Y2H ‘gold standard’ assays. We retested a subset of ProCREate detected PPIs doing tradition 1x1 Y2H and confirmed 72%, suggesting replicates detected ~60% of all AIrepeat predicted PPIs. While ProCREate has the potential to detect 100% of predicted PPIs by increasing replicates and/or mating efficiency, we are moving on to screen genome size *Arabidopsis* libraries to estimate assay capacity. We will then use ProCREate to screen cDNA libraries made from feedstocks by shotgun cloning into our Y2H plasmids. PPI data generated will yield deeper insight into many molecular processes and pathways, enabling these interactomes to be used to guide improvement of feedstock productivity and sustainability.

References

1. *Arabidopsis* Interactome Mapping Consortium, Science 333, 6042 (2011).

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