140. Creating a Multi-Functional Library of Grass Transcription Factors for the Energy Crop Model System *Brachypodium distachyon*

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Project Goals: The overall goal of this project is to develop BradiTORFL, a comprehensive *Brachypodium distachyon* Transcription Factor ORF Library.

Comprehensive collections of full-length transcription factor cDNAs (fl-cDNA) have proven to be an extraordinary reagent for advanced research systems such as human, *Caenorhabditis elegans*, *Drosophila melanogaster*, and *Arabidopsis thaliana*. One of the seven DOE-JGI flagship plant genome species, *Brachypodium distachyon*, serves as a model for potential energy crops such as switchgrass, sorghum, and *Miscanthus*, as well as for the cereal crops that constitute a large part of the world's diet. We are constructing a complete grass transcription factor collection in an entry vector that will be of value for numerous functional studies. Even with a strong history of resource development that included numerous large-scale multinational projects, the *A. thaliana* collection lacks approximately 200 genes predicted to bind DNA. To overcome these same pitfalls and accelerate reagent development, we have included gene synthesis as a technique to capture fl-cDNAs. Through the analysis of several types of expression profiling data sets, we identified high priority candidates for the regulation of biofuel feedstock relevant traits, such as growth and cell wall biosynthesis and abiotic stress tolerance. The DOE-JGI synthesized 143 unique transcription factors from families that include bHLH, bZIP, CCAAT, GRAS, Homeodomain, HSF, MADS box, MYB, NAM, WRKY, and several classes of zinc fingers. Specific subfamilies include putative Aux/IAA-ARF auxin response factors, bHLH factors predicted to function in light signaling, G-box binding proteins implicated in light signaling, CBF-like genes predicted to function in abiotic stress responses, ethylene- associated factors, GRAS type genes implicated in growth and development, and MYBs predicted to function in cell wall, circadian clock and light signaling, and CCT domain containing genes associated with circadian clock and photoreceptor signaling. The collection will be transferred into multiple destination vectors for downstream applications including protein- DNA and protein-protein interaction platforms in yeast. Genes that yield positive interactions can then be shuttled from their pENTR vector to a variety of other constructs for various purposes including expression *in planta* to further characterize their functions. We are presently evaluating *B. distachyon* protein-DNA interactions in yeast using two approaches. The first is "gene-centric" where a promoter is tested for interactions with all transcription factor proteins in the library. The second approach, developed by the Mockler Lab, is "protein-centric" and interrogates the capacity of each transcription factor protein to interact with a collection of 768 synthetic 250 bp promoters. This collection of synthetic promoters was designed to maximize potential binding motif sequence diversity and all possible 8 nt DNA motifs occur in at least 4 independent promoters. Proof-of-concept experiments demonstrate the utility of this approach and we are currently expanding the analysis to infer the binding specificities for all of the transcription factors synthesized by DOE-JGI in this project.

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