Essential Genes with Unknown Functions in *Arabidopsis*

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Many genes in *Arabidopsis* are currently annotated to encode proteins with unknown functions. An important goal of current genomics research is to determine what cellular functions these proteins perform. In our SeedGenes Project (www.seedgenes.org), we are studying genes that are essential for seed development. More than 20% of the genes in the evolving SeedGenes database appear to lack established protein motifs and are predicted to encode proteins with unknown functions. These genes represent a valuable subset of unknowns suitable for further study because they are known to be essential. We are taking two complementary approaches with these genes: (1) isolating other mutant alleles through reverse genetics to increase our confidence that the correct gene has been identified; and (2) identifying interacting proteins using the tandem affinity purification (TAP) tagging method.

In order to meet the first objective, we have obtained putative duplicate alleles from the Salk collection of insertion lines and established protocols for using genetic complementation tests, flanking sequence data, and PCR-based cosegregation analysis to confirm that the correct gene has been identified. These confirmation steps are particularly important with unknowns because one cannot use the predicted protein function to conclude that a logical candidate for an essential gene has been identified.

We chose TAP tagging over other methods of identifying interacting proteins because it has proved very useful in yeast and because it facilitates identification of interactions within the host organism, potentially at endogenous protein levels. Seven genes selected for initial studies have been amplified along with their promoter regions from wild-type plants. TAP-tags were added using overlap PCR and the resulting constructs were transferred with a binary vector into *Arabidopsis* plants, both wild type and heterozygous. An overview of results obtained to date will be presented. Recovery of TAP-tagged proteins and characterization of interacting partners by mass spectrometry will be used in the future to help decipher the cellular functions of these essential gene products.

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