HIGH-THROUGHPUT SCREENING STRATEGIES FOR NOVEL HERBICIDE TARGETS. Cory A. Christensen, Adel M. Zayed, Lining Guo, Kurt Boudonck, Todd M. DeZwaan, Rao V. Mulpuri, Vereesh L. Sevala, and Keith R. Davis, Research Scientist, Staff Scientist, Director of Biochemistry, Research Scientist, Research Scientist, Senior Research Scientist, Staff Scientist, and Vice President of Agricultural Research, Paradigm Genetics, Inc., Research Triangle Park, NC 27709.

We have developed a high-throughput screen for novel herbicide targets. The objective of this screen is to identify essential plant enzymes that can serve as targets in high-throughput screens for chemical compounds with herbicidal activity. To identify herbicide target genes we use the weed *Arabidopsis thaliana*. Essential *A. thaliana* genes are identified by screening for lethal phenotypes in lines where the target gene has been inactivated by anti-sense RNA expression. We use two methods of transgene expression, direct expression and transactivation. In the direct expression system, the anti-sense message is driven by a constitutive promoter. We screen for lethal phenotypes in the T1 generation and analyze multiple independent transformation events per target gene. In the transactivation system, expression of the anti-sense message depends on the independent expression of a transcriptional activator, or driver, that binds to an activating sequence upstream from the transgene. In this screen, the target gene construct and driver are brought together by crossing a driver-containing line and a T1 generation target-containing line. We screen for lethal phenotypes among the F1 progeny from multiple transformation events per target gene.

Anti-sense inactivation of an essential *A. thaliana* gene results in a lethal phenotype that severely impacts the health or viability of the seedling. Lethal phenotypes include chlorosis, necrosis, reduced plant size, and severe developmental abnormalities. If one or more of these phenotypes are seen in multiple individuals from independent transformation events, the target gene is promoted for assay development.

The purpose of assay development is to generate a method for screening the target gene function that is sufficiently robust for high throughput or ultra-high throughput screening applications. A thorough literature review and functional analysis of the target gene sequence is performed to determine the most appropriate assay format. Assay formats that we routinely use to evaluate protein function include *in vitro* assays of enzymatic functions, substrate binding assays, and cell-based assays in which the target gene is expressed in a surrogate host.

Once an assay has been developed, the target gene activity is tested against a library of tens of thousands of chemical compounds to identify ones capable of inhibiting the reaction. Hits from this ultra-high throughput screening are further tested on plants for *in vivo* herbicidal activity. At this point, these chemical compounds constitute product leads in an herbicide discovery program.

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