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Use of *CRISPRi* for rapid characterization of a soybean (*Glycine max*) *GmScream* promoter

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Gene expression undergirds the growth, development, and all agronomically important traits of soybean (*Glycine max*). Each soybean gene is tunable by a distinct promoter region, located upstream of the gene coding region. To better understand the roles of promoters in gene expression, we developed and optimized a rapid promoter functional analysis tool, utilizing *CRISPR* interference (*CRISPRi*). In a proof-of-concept study targeting the strong, constitutive soybean *GmScreamM8* promoter, deactivated *Cas9* protein (*dCas9*) was directed by sequence-specific guide RNAs (*gRNA*) to bind to various targeted sites in the promoter during transient gene expression in lima bean (*Phaseolus lunatus*) cotyledon tissue. Gene transcription was altered as *dCas9* proteins competitively displaced active DNA-binding proteins. For DNA introduction, a promoter-*green fluorescent protein* (*gfp*) fusion was co-introduced along with *gRNA* expression and *dCas9*expression constructs into cotyledon tissues through particle bombardment. Changes in the transcription of the *gfp* reporter gene due to *CRISPRi* were determined by measuring changes in fluorescence intensity over 60 - 120 hours. Modification of the ratio of promoter-*gfp* fusion construct to the *gRNA* and *dCas9* constructs during co-bombardment resulted in significantly improved detection of *CRISPRi* effects. A total of 10 *gRNA* targets were assessed, consisting of 1 plasmid backbone target, 8 *GmScreamM8* promoter targets, and 1 *gfp* coding sequence target. *CRISPRi* targeting of sites within *GmScreamM8* allowed for mapping of important motifs within the promoter, which contributed to gene expression. Notably, our *CRISPRi* approach maintains the original promoter sequence, while other previous promoter functional characterization approaches rely on altering the promoter sequence to identify important motifs. These findings represent the first documented confirmation of the use of *CRISPRi* for promoter characterization in plants.