PROOF-OF-CONCEPT IMPLEMENTATION OF A GENETIC NOR GATE USING TRANSLATIONAL REPRESSION IN TOBACCO

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Functional sensing—which relies on direct monitoring of plant health using plants' genotypic response—has been proposed as a promising alternative to conventional sensing technologies used in agriculture, with proofs-of-concept demonstrating high reliability for detection of environmental stresses [1]–[4]. Such systems generally rely on rudimentary synthetic biology approaches, linking a single inducible promoter sensitive to the stimulus of interest with a reporter gene—commonly a fluorescent protein [5]–[7]. The overlap in the emission ranges of such fluorescent proteins limits the number of proteins which can be used in a single sensing system. Combined with the one-to-one mapping between input stimuli and reporter proteins, this severely limits the scalability of functional sensing systems.

Recent advances in synthetic biology allow for implementation of genetic circuits which perform Boolean computations in-vivo [8]–[12]. Applied to functional sensing, this can allow for reporting on multiple stimuli using a single reporter protein. Of the few implementations of genetic circuits in plants, most focus on modifying plant behaviour rather than sensing, and rely on irreversible genetic changes, making them ill-suited for sensing applications.

We present a proof-of-concept realization of a fully-reversible genetic logic circuit in tobacco plants, based on translational repression. A Cas6-based translational repression system [13], [14] is used to implement a 2-input NOR gate (Fig. 1) with exposure to estradiol and to ethanol serving as inputs, and expression of green fluorescent protein (GFP) serving as the output. On exposure to a stimulus, the corresponding inducible promoter is activated, leading to the expression of the corresponding Cas protein. The Cas proteins then bind upstream of the GFP coding sequence and cleave the mRNA, resulting in repression of GFP translation, thus resulting in reduced expression of GFP in the presence of at least one of the stimuli.

Tobacco plants were transiently transformed with the designed genetic constructs, exposed to ethanol and estradiol, and imaged using a confocal microscope. The images were analysed to quantify the extent of GFP expression as a function of the input stimuli. The expression of GFP was observed to be significantly (two-fold to four-fold) higher in control samples, as compared to samples exposed to at least one inducer.

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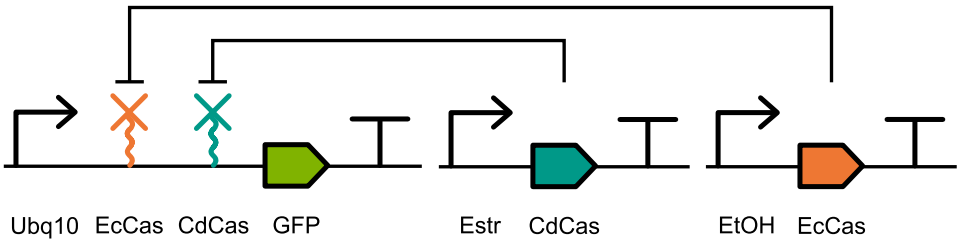

FIGURE 1. Schematic representation of genetic constructs and interactions used for realization of a NOR gate

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